



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : G01N 33/564, 33/569		A1	(11) International Publication Number: WO 99/60403 (43) International Publication Date: 25 November 1999 (25.11.99)
<p>(21) International Application Number: PCT/US99/10371</p> <p>(22) International Filing Date: 11 May 1999 (11.05.99)</p> <p>(30) Priority Data: 09/079,435 15 May 1998 (15.05.98) US</p> <p>(71) Applicant: PROMETHEUS LABORATORIES, INC. [US/US]; 5739 Pacific Center Boulevard, San Diego, CA 92121 (US).</p> <p>(72) Inventors: WALSH, Michael, J.; 1945 Willow Lane, San Diego, CA 92106 (US). ROSE, Steven, L.; 3207 Avenida Reposo, Escondido, CA 92029 (US).</p> <p>(74) Agents: GASHLER, Andrea, L. et al.; Campbell & Flores LLP, Suite 700, 4370 La Jolla Village Drive, San Diego, CA 92122 (US).</p>		<p>(81) Designated States: AU, CA, IL, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p>Published <i>With international search report.</i></p>	
<p>(54) Title: INFLAMMATORY BOWEL DISEASE FIRST STEP ASSAY SYSTEM</p> <p>(57) Abstract</p> <p>The present invention provides a highly sensitive method of diagnosing inflammatory bowel disease (IBD) in an individual. The method includes the steps of isolating a sample from the individual; determining by non-histological means whether the sample is positive for anti-neutrophil cytoplasmic antibodies (ANCA); determining whether the sample is positive for anti-<i>Saccharomyces cerevisiae</i> immunoglobulin A (ASCA-IgA); determining whether the sample is positive for anti-<i>Saccharomyces cerevisiae</i> immunoglobulin G (ASCA-IgG); and diagnosing the individual as having IBD when the sample is positive for ANCA, ASCA-IgA or ASCA-IgG, and diagnosing the individual as not having IBD when the sample is negative for ANCA, ASCA-IgA and ASCA-IgG, provided that the method does not include histological analysis of neutrophils.</p>			

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IR	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		

INFLAMMATORY BOWEL DISEASE FIRST STEP ASSAY SYSTEM**BACKGROUND OF THE INVENTION****FIELD OF THE INVENTION**

The invention relates generally to the fields
5 of inflammatory bowel disease and immunology and more
specifically to serological methods for distinguishing
inflammatory bowel disease from other disorders.

BACKGROUND INFORMATION

Inflammatory bowel disease (IBD), which occurs
10 world-wide and afflicts millions of people, is the
collective term used to describe two gastrointestinal
disorders of unknown etiology: Crohn's disease (CD) and
ulcerative colitis (UC). IBD together with irritable
bowel syndrome (IBS) will affect one-half of all
15 Americans during their lifetime, at a cost of greater
than \$2.6 billion dollars for IBD and greater than \$8
billion dollars for IBS. A primary determinant of these
high medical costs is the difficulty of diagnosing
digestive diseases. The cost of IBD and IBS is
20 compounded by lost productivity, with persons suffering
from these disorders missing at least 8 more days of work
annually than the national average.

Inflammatory bowel disease has many symptoms in
common with irritable bowel syndrome, including abdominal
25 pain, chronic diarrhea, weight loss and cramping, making
definitive diagnosis extremely difficult. Of the 5
million people suspected of suffering from IBD in the
U.S., only 1 million are diagnosed as such. The
difficulty in differentially diagnosing IBD and IBS

hampers early and effective treatment of these diseases. Thus, there is a need for rapid and sensitive testing methods for definitively distinguishing IBD from IBS.

Progress has been made in precisely diagnosing, 5 in many cases, Crohn's disease and ulcerative colitis. However, current methods for diagnosing an individual as having Crohn's disease or ulcerative colitis, while highly specific, are relatively costly, requiring labor intensive immunofluorescence assays and careful analysis 10 of cell staining patterns. Although these costly assays are easily justified for those individuals previously diagnosed with or strongly suggested to have IBD, a less expensive but highly sensitive alternative would be advantageous for first determining if an individual has 15 inflammatory bowel disease at all. Such a highly sensitive primary screening assay would provide physicians with an inexpensive means for rapidly distinguishing individuals with IBD from those having IBS, thereby facilitating earlier and more appropriate 20 therapeutic intervention and minimizing uncertainty for patients and their families. If desired, such a primary screening assay could be combined with a subsequent, highly specific assay for determining if an individual diagnosed with IBD has Crohn's disease or ulcerative 25 colitis.

Unfortunately, such a highly sensitive and inexpensive primary screening assay for distinguishing IBD from other digestive diseases presenting with similar symptoms is currently not available. Thus, there is a 30 need for a method of rapidly diagnosing inflammatory bowel disease at a very early stage of disease progression. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

The present invention provides a highly sensitive method of diagnosing inflammatory bowel disease (IBD) in an individual. The method includes the steps of 5 isolating a sample from the individual; determining by non-histological means whether the sample is positive for anti-neutrophil cytoplasmic antibodies (ANCA); determining whether the sample is positive for anti-*Saccharomyces cerevisiae* immunoglobulin A 10 (ASCA-IgA); determining whether the sample is positive for anti-*Saccharomyces cerevisiae* immunoglobulin G (ASCA-IgG); and diagnosing the individual as having IBD when the sample is positive for ANCA, ASCA-IgA or ASCA-IgG, and diagnosing the individual as not having IBD 15 when the sample is negative for ANCA, ASCA-IgA and ASCA-IgG, provided that the method does not include histological analysis of neutrophils. In a method of the invention, ANCA, ASCA-IgA or ASCA-IgG positivity can be conveniently determined, for example, using an 20 immunoassay.

The present invention further provides a highly sensitive method of diagnosing IBD in an individual. This method of the invention includes the steps of isolating a sample from the individual; determining by 25 non-histological means whether the sample has an ANCA level above an ANCA cut-off value (X); determining whether the sample has an ASCA-IgA level above an ASCA-IgA cut-off value (Y); determining whether the sample has an ASCA-IgG level above an ASCA-IgG cut-off 30 value (Z); and diagnosing the individual as having IBD when the ANCA level is above X, the ASCA-IgA level is above Y, or the ASCA-IgG level is above Z, and diagnosing the individual as not having IBD when the ANCA level is

below X, the ASCA-IgA level is below Y, and the ASCA-IgG value is below Z, where X, Y, and Z are independently selected to achieve optimized sensitivity, specificity, negative predictive value, positive predictive value or 5 overall agreement, provided that the method does not include histological analysis of neutrophils.

In a highly sensitive method of diagnosing IBD provided by the present invention, X, Y and Z can be independently selected such that, for example, the 10 sensitivity of diagnosing an individual with IBD is at least about 70%, and can be selected such that, additionally, the specificity of diagnosing an individual with IBD is 30-60%. In addition, X, Y and Z can be independently selected such that the sensitivity of 15 diagnosing an individual with IBD is at least about 70%, the specificity of diagnosing an individual with IBD is 30-60%, and the negative predictive value in a population having an IBD disease prevalence of about 15% is at least about 90% and can be, for example, at least about 95%.

20 Furthermore, X, Y and Z can be independently selected such that the sensitivity of diagnosing an individual with IBD is at least about 90%, and can be selected such that, additionally, the specificity of diagnosing an individual with IBD is 20-60%. If desired, 25 X, Y and Z can be independently selected such that the sensitivity of diagnosing an individual with IBD is at least about 90%, the specificity of diagnosing an individual with IBD is 20-60%, and the negative predictive value in a population having an IBD disease 30 prevalence of about 15% is at least about 90%. The negative predictive value can be, for example, at least

about 95%. In addition, X, Y and Z can be independently selected such that, for example, the sensitivity of diagnosing an individual with IBD is about 90%, the specificity is about 37%, and the negative predictive 5 value in a population having an IBD disease prevalence of about 15% is at least about 95%. In one embodiment, X can be selected to be 0.7 multiplied by two standard deviations above the background value of ANCA-negative UC sera, Y can be selected to be 12 ELISA units, and Z can be 10 selected to be 60 ELISA units.

In a method of the invention for diagnosing inflammatory bowel disease, the ANCA, ASCA-IgA and ASCA-IgG levels can be determined using, for example, a serum sample or saliva sample. ANCA levels can be 15 determined using an antigen specific for ANCA such as fixed neutrophils, and ASCA-IgA and ASCA-IgG levels can be determined using an antigen specific for ASCA such as yeast cell wall phosphopeptidomannan (PPM), which can be prepared, for example, from strain ATCC #38926.

20 The invention additionally provides a highly efficient method of analyzing multiple samples for IBD by first assaying all samples for the presence or absence of ANCA; next assaying only ANCA-negative samples for the presence or absence of ASCA-IgA; and next assaying only 25 ANCA-negative and ASCA-IgA-negative samples for the presence or absence of ASCA-IgG, where the presence of pANCA, ASCA-IgA or ASCA-IgG in a sample is indicative of IBD and where the absence of ANCA, ASCA-IgA and ASCA-IgG is indicative of the absence of IBD. In such a method of 30 the invention, the presence of ANCA, ASCA-IgA and ASCA-IgG can be conveniently determined, for example, using an immunoassay.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the IBD First Step central composite design.

DETAILED DESCRIPTION OF THE INVENTION

5 The present invention is directed to the discovery that three enzyme-linked immunosorbent assays (ELISAs) can be combined without immunofluorescence or other histological analysis of neutrophils to diagnose inflammatory bowel disease (IBD) with high sensitivity.

10 In particular, as disclosed in Example II, an ELISA assay for anti-neutrophil cytoplasmic antibodies (ANCA), an ELISA assay for anti-*Saccharomyces cerevisiae* immunoglobulin A (ASCA-IgA) and an ELISA assay for anti-*Saccharomyces cerevisiae* immunoglobulin G (ASCA-IgG)

15 were combined to produce a highly sensitive means of distinguishing individuals having either Crohn's disease (CD) or ulcerative colitis (UC) from other individuals, such as those having irritable bowel syndrome (IBS). Such a method does not include labor intensive

20 immunofluorescence analysis of fixed neutrophils or other neutrophil histological analysis. Thus, the methods of the invention provide a rapid and sensitive means of differentiating individuals having either CD or UC from those who do not have IBD. The methods of the invention

25 can be used alone to rule out inflammatory bowel disease in an individual suspected of having the disease, or, when positive for diagnosis of IBD, can be used in combination with a subsequent assay that specifically differentiates CD from UC.

Thus, the present invention provides a highly sensitive method of diagnosing IBD in an individual. The method includes the steps of isolating a sample from the individual; determining by non-histological means whether 5 the sample is positive for ANCA; determining whether the sample is positive for ASCA-IgA; determining whether the sample is positive for ASCA-IgG; and diagnosing the individual as having IBD when the sample is positive for ANCA, ASCA-IgA or ASCA-IgG, and diagnosing the individual 10 as not having IBD when the sample is negative for ANCA, ASCA-IgA and ASCA-IgG, provided that the method does not include histological analysis of neutrophils. In a method of the invention, ANCA, ASCA-IgA and ASCA-IgG positivity can be determined, for example, using an 15 immunoassay.

As used herein, the term "inflammatory bowel disease" is synonymous with "IBD" and is a collective term referring to both Crohn's disease and ulcerative colitis. Thus, an individual having either Crohn's 20 disease or ulcerative colitis is defined herein as having IBD. Conversely, an individual having neither ulcerative colitis nor Crohn's disease does not have IBD as defined herein. The term "inflammatory bowel disease" distinguishes Crohn's disease and ulcerative colitis from 25 all other disorders, syndromes or abnormalities of the gastroenterological tract including irritable bowel syndrome.

The methods of the invention for diagnosing IBD involve determining whether a sample is positive for 30 anti-neutrophil cytoplasmic antibodies (ANCA). Anti-neutrophil cytoplasmic antibodies that produce a perinuclear staining pattern (pANCA) are elevated in 60-80% of UC patients and less frequently in CD and other

disorders of the colon. Serum titers of ANCA are elevated in UC patients regardless of clinical status and, thus, do not reflect disease activity. High levels of serum ANCA also persist in UC patients five years 5 post-colectomy. Although pANCA is found only very rarely in healthy adults and children, healthy relatives of UC patients have an increased frequency of pANCA, indicating that pANCA may be an immunogenetic susceptibility marker. ANCA reactivity is also present in a small portion of 10 patients with Crohn's disease. The reported prevalence in CD varies, with most studies reporting that 10 to 30% of CD patients express ANCA (Saxon et al., J. Allergy Clin. Immunol. 86:202-210 (1990); Cambridge et al., Gut 33:668-674 (1992); Pool et al., Gut 34:46-50 (1993); and 15 Brokroelofs et al., Dig. Dis. Sci. 39:545-549 (1994)).

As used herein, the term "anti-neutrophil cytoplasmic antibody" is synonymous with "ANCA" and means antibodies to cytoplasmic components of a neutrophil. ANCA, such as serum or saliva ANCA, can be detected using 20 an enzyme-linked immunosorbent assay with alcohol-fixed neutrophils, for example (see Example I). As disclosed herein, ANCA activity is divided into several broad categories: perinuclear to nuclear staining or cytoplasmic staining with perinuclear highlighting 25 (pANCA); cytoplasmic neutrophil staining without perinuclear highlighting (cANCA); and diffuse staining with speckling across the entire neutrophil (SAPPA). The term ANCA, as used herein, encompasses all varieties of anti-neutrophils cytoplasmic reactivity, including pANCA, 30 cANCA and SAPPA. Similarly, the term "ANCA" encompasses all immunoglobulin isotypes including, for example, immunoglobulin A and G.

The determination of whether a sample is positive for ANCA using non-histological means is made using antigen specific for ANCA. Such an antigen specific for ANCA can be, for example, whole fixed neutrophils; an unpurified or partially purified neutrophil extract; a purified UC pANCA antigen such as a purified protein, protein fragment or synthetically produced peptide; an anti-ANCA idiotypic antibody; or the like. Particularly useful antigens specific for ANCA are peptides, which can be chemically synthesized or expressed on the surface of phage. Purified antigens specific for ANCA can be, for example, histone H1, or an ANCA-reactive fragment of histone H1, as described in U.S. application Serial No. 08/837,058; an ulcerative colitis pANCA secretory vesicle antigen or an ANCA-reactive fragment thereof, as described in U.S. application Serial No. 08/804,106; or a microbial UC pANCA antigen, such as a histone H1-like antigen, porin antigen, Bacteroides antigen, or ANCA-reactive fragment thereof, as described in U.S. application Serial No. 09/041,889. One skilled in the art understands that additional antigens specific for ANCA, including antigenic fragments and ANCA-reactive peptides, can be identified, for example, using a representative UC pANCA monoclonal antibody, such as one described in U.S. application Serial No. 08/472,688.

In the methods of the invention, a sample to be analyzed is obtained from the individual to be diagnosed. The term "sample," as used herein, means any biological specimen obtained from an individual that contains antibodies. A sample can be, for example, whole blood, plasma, saliva or other bodily fluid or tissue having antibodies, preferably a serum sample. Preferably, although not necessarily, a sample contains both ANCA and

ASCA antibodies. The use of a serum sample is described in Example I; the use of other samples, such as saliva and urine samples, is well known in the art (see, for example, Hashida et al., J. Clin. Lab. Anal. 11:267-86 5 (1997), which is incorporated by reference herein). One skilled in the art understands that samples such as serum samples can be diluted prior to analysis of ANCA, ASCA-IgA and ASCA-IgG content.

10 The methods of the invention for diagnosing IBD also involve determining whether a sample is positive for immunoglobulin A anti-*Saccharomyces cerevisiae* antibodies (ASCA-IgA) or immunoglobulin G anti-*Saccharomyces cerevisiae* antibodies (ASCA-IgG). Previous reports 15 indicate that such antibodies can be elevated in patients having Crohn's disease, although the nature of the *S. cerevisiae* antigen supporting the specific antibody response in CD is unknown (Sendid et al., Clin. Diag. Lab. Immunol. 3:219-226 (1996), which is incorporated 20 herein by reference). ASCA may represent a response against yeasts present in common food or drink or a response against yeasts that colonize the gastrointestinal tract. Studies with periodate oxidation have shown that the epitopes recognized by ASCA in CD 25 patient sera contain polysaccharides. Oligomannosidic epitopes are shared by a variety of organisms including different yeast strains and genera, filamentous fungi, viruses, bacteria and human glycoproteins. Thus, mannose-induced antibody responses in CD may represent a 30 response against a pathogenic yeast organism or against a cross-reactive oligomannosidic epitope present, for example, on a human glycoprotein autoantigen. Regardless of the nature of the antigen, elevated levels of serum ASCA are believed to be a differential marker for Crohn's

disease, with only low levels of ASCA reported in UC patients (Sendid et al., *supra*, 1996).

As used herein, the term "anti-*Saccharomyces cerevisiae* immunoglobulin A" is synonymous with 5 "ASCA-IgA" and refers to antibodies of the immunoglobulin A isotype that react specifically with *S. cerevisiae*. Similarly, the term "anti-*Saccharomyces cerevisiae* immunoglobulin G" is synonymous with "ASCA-IgG" and refers to antibodies of the immunoglobulin G isotype that 10 react specifically with *S. cerevisiae*. The determination of whether a sample is positive for ASCA-IgA or ASCA-IgG is made using an antigen specific for ASCA. Such an antigen can be any antigen or mixture of antigens that is bound specifically by immunoglobulin A ASCA or 15 immunoglobulin G ASCA. Although ASCA antibodies were initially characterized by their ability to bind *S. cerevisiae*, those of skill in the art will understand that an antigen that is bound specifically by ASCA can be obtained from *S. cerevisiae*, or can be obtained from a 20 variety of other sources so long as the antigen is capable of binding specifically to ASCA antibodies. Accordingly, exemplary sources of an antigen specific for ASCA, which can be used to determine whether a sample is positive for ASCA-IgA or ASCA-IgG, include whole killed 25 yeast cells, such as *Saccharomyces* or *Candida* cells; yeast cell wall phosphopeptidomannan (PPM); oligomannosides; neoglycolipids; anti-ASCA idiotypic antibodies; and the like. As described above, different species and strains of yeast, including *Saccharomyces*, 30 can be an antigen specific for ASCA useful for determining whether a sample is positive for ASCA-IgA or ASCA-IgG. For example, *S. cerevisiae* strain Sul, Su2, CBS 1315 or BM 156, or *Candida albicans* strain VW32, can

be used as an antigen specific for ASCA in a method of the invention.

Preparations of yeast cell wall mannans, or phosphopeptidomannans (PPM), are can be used to determine 5 if a sample is positive for ASCA-IgA or ASCA-IgG. Such water soluble surface antigens can be prepared by appropriate extraction techniques, including autoclaving as described in Example I, or can be obtained commercially (see Lindberg et al., Gut 33:909-913 (1992), 10 which is incorporated herein by reference). The acid stable fraction of yeast cell wall PPM also can be useful in the methods of the invention (Sendid et al., *supra*, 1996). An exemplary PPM that is useful in determining whether a sample is positive for ASCA-IgA or ASCA-IgG is 15 derived from *S. cerevisiae* strain ATCC #38926.

Purified oligosaccharide antigens, such as oligomannosides, also can be useful in determining whether a sample is positive for ASCA-IgA or ASCA-IgG in a method of the invention. For use herein, the purified 20 oligomannoside antigens are preferably converted into neoglycolipids as described in Faille et al., Eur. J. Microbiol. Infect. Dis. 11:438-446 (1992). One skilled in the art understands that the reactivity of such an oligomannoside antigen with ASCA can be optimized by 25 varying the mannosyl chain length (Frosh et al., Proc. Natl. Acad. Sci. USA, 82:1194-1198 (1985)); the anomeric configuration (Fukazawa et al., *In* E. Kurstak (ed.), Immunology of Fungal Disease, Marcel Dekker Inc., New York, pp. 37-62 (1989); Nishikawa et al., Microbiol. Immunol., 34:825-840 (1990); Poulain et al., Eur. J. Clin. Microbiol., 23:46-52 (1993); Shibata et al., Arch. Biochem. Biophys., 243:338-348 (1985); and Trinel et al., Infect. Immun., 60:3845-3851 (1992)); or the position of 30

the linkage (Kikuchi et al., Planta, 190:525-535 (1993)). Each of the foregoing references are incorporated herein by reference in their entirety.

An antigen specific for ASCA useful in
5 determining whether a sample is positive for ASCA-IgA or
ASCA-IgG can be, for example, an oligomannoside which
includes the mannotetraose Man(1-3)Man(1-2) Man(1-2)Man.
Such an oligomannoside can be purified from PPM as
described in Faille et al., *supra*, 1992. An exemplary
10 neoglycolipid which is an antigen specific for ASCA can
be constructed by releasing the oligomannoside from its
respective PPM and subsequently coupling the released
oligomannoside to 4-hexadecylaniline or the like.

Prior to the present invention, ANCA and ASCA
15 analysis have been combined in order, for example, to
increase the specificity of an assay for differentiating
UC from Crohn's disease or to determine clinical subtypes
of CD (Quinton et al., Gastroenterol. 112:A1066 (1997);
Seidman et al., Gastroenterol. 112:A1087 (1997); and
20 Vasiliauskas et al., Gastroenterol. 112:A1112 (1997)).
In contrast, the methods of the present invention, which
are of high sensitivity, are directed to determining if
an individual has either UC or CD but do not distinguish
between the two diseases. Thus, the methods of the
25 invention are useful, for example, to sensitively
distinguish between IBD and other digestive disorders
such as irritable bowel syndrome and infectious digestive
diseases and, when positive for IBD, can be used in
conjunction, if desired, with a subsequent specific assay
30 in order to precisely determine whether the individual
with IBD has UC or CD. Furthermore, previous studies in
which ANCA analysis has been combined with analysis of
ASCA-IgA and ASCA-IgG have additionally included

immunofluorescence of fixed neutrophils in order to determine the type of ANCA reactivity present (see Quinton et al., *supra*, 1997; Seidman et al., *supra*, 1997; Dubinsky et al., *supra*, 1997; and Vasiliauskas et al., *supra*, 1997). In contrast to these studies, the methods of the present invention explicitly exclude histological analysis of neutrophils.

Thus, the present invention is directed to a highly sensitive method of diagnosing inflammatory bowel disease, which does not include histological analysis of neutrophils. As used herein, the term "histological analysis of neutrophils" means any technique revealing the structure of a neutrophilic cell using staining or microscopy. Histological analysis, which encompasses techniques such as immunocytochemistry and indirect immunofluorescence, as well as other methods involving microscopy, is explicitly excluded from the present invention. In contrast, an enzyme-linked immunosorbent assay (ELISA), in which neutrophil reactivity is analyzed by means of a detectable secondary antibody that generates a quantitative signal, does not involve microscopy or other analysis of cell structure and, therefore, is not "histological analysis of neutrophils" as defined herein.

As further disclosed herein, three ELISA cut-off values for determining if a sample is positive or negative for ANCA ("X"), ASCA-IgA ("Y") and ASCA-IgG ("Z") were simultaneously varied using Factorial Design Optimization to achieve a desired degree of sensitivity (Example II). Using this approach, cooperative interactions among the ANCA, ASCA-IgA and ASCA-IgG cut-off values were identified. For example, particular ANCA, ASCA-IgA and ASCA-IgG cut-off values were

determined to diagnose an individual with IBD with greater than about 90% sensitivity, which is a greater than 90% probability that an individual having IBD by colonoscopic, radiologic and/or histologic criteria would 5 be diagnosed as such. Thus, the present invention provides a method of diagnosing inflammatory bowel disease with a greater sensitivity than previously available. Similarly, using Factorial Design Optimization, for example, other ANCA, ASCA-IgA and 10 ASCA-IgG cut-off values can be determined which provide a clinically useful sensitivity, specificity, negative predictive value, positive predictive value or overall agreement for a particular patient population. If desired, one can select the ANCA, ASCA-IgA and ASCA-IgG 15 cut-off values "X," "Y," and "Z" to give a desired sensitivity combined with, for example, a desired specificity and negative predictive value.

The present invention therefore provides a highly sensitive method of diagnosing IBD in an 20 individual by isolating a sample from the individual; determining by non-histological means whether the sample has an ANCA level above an ANCA cut-off value (X); determining whether the sample has an ASCA-IgA level above an ASCA-IgA cut-off value (Y); determining whether 25 the sample has an ASCA-IgG level above an ASCA-IgG cut-off value (Z); and diagnosing the individual as having IBD when the ANCA level is above X, the ASCA-IgA level is above Y, or the ASCA-IgG level is above Z, and diagnosing the individual as not having IBD when the ANCA 30 level is below X, the ASCA-IgA level is below Y, and the ASCA-IgG value is below Z, where X, Y, and Z are independently selected to achieve an optimized sensitivity, specificity, negative predictive value, positive predictive value or overall agreement, provided.

that the method does not include histological analysis of neutrophils.

As used herein, the term "X" refers to an ANCA cut-off value, against which an experimental ANCA sample 5 value is compared. Similarly, as used herein, the term "Y" refers to an ASCA-IgA cut-off value, against which an experimental ASCA-IgA value is compared. The term "Z," as used herein, refers to an ASCA-IgG cut-off value, against which an experimental ASCA-IgG cut-off value is 10 compared. As disclosed herein, when an ANCA level is above X, or an ASCA-IgA level is above Y, or an ASCA-IgG level is above Z, an individual is diagnosed as having IBD.

The clinical parameters of sensitivity, 15 specificity, negative predictive value, positive predictive value and overall agreement are calculated using true positives, false positives, false negatives and true negatives. A "true positive" sample is a sample positive for IBD according to colonoscopic, radiologic 20 and/or histologic analysis, which is also diagnosed positive according to a method of the invention. A "false positive" sample is a sample negative for IBD by colonoscopic, radiologic and/or histologic analysis, which is diagnosed positive according to a method of the 25 invention. Similarly, a "false negative" is a sample positive for IBD by colonoscopic, radiologic and/or histologic analysis, which is diagnosed negative according to a method of the invention. A "true negative" is a sample negative for IBD by colonoscopic, 30 radiologic and/or histologic analysis, and also negative for IBD according to a method of the invention. See, for example, Motulsky (Ed.), Intuitive Biostatistics New

York: Oxford University Press (1995), which is incorporated herein by reference.

As used herein, the term "sensitivity" means the probability that a laboratory method is positive in the presence of IBD. Sensitivity is calculated as the number of true positive results divided by the sum of the true positives and false negatives. Sensitivity essentially is a measure of how well a method correctly identifies those with disease. In a method of the invention, the ANCA, ASCA-IgA, and ASCA-IgG values can be selected such that the sensitivity of diagnosing an individual is at least about 60%, and can be, for example, at least about 65%, 70%, 75%, 80%, 85%, 90% or 95%. As illustrated in Example II, the maximum sensitivity of diagnosing IBD using a method of the invention is about 96.5%. A method of diagnosing IBD in an individual is particularly useful when the sensitivity is at least about 80%, or at least about 90%.

As used herein, the term "specificity" means the probability that a method is negative in the absence of IBD. Specificity is calculated as the number of true negative results divided by the sum of the true negatives and false positives. Specificity essentially is a measure of how well a method excludes those who do not have IBD. In a method of the invention, the ANCA, ASCA-IgA, and ASCA-IgG cut-off values "X," "Y," and "Z" can be selected such that, when the sensitivity is at least about 70%, the specificity of diagnosing an individual is in the range of 30-60%, for example, 35-60%, 40-60%, 45-60% or 50-60%. Furthermore, in a method of the invention, the ANCA, ASCA-IgA, and ASCA-IgG cut-off values "X," "Y," and "Z" can be selected such that, when the sensitivity is at least about 90%, the

specificity of diagnosing an individual is in the range of 20-60%, for example, 20-30%, 20-40%, 20-50%, 30-60%, 35-60%, 40-60%, 45-60% or 50-60%. As illustrated in Example II, the maximum specificity that can be obtained 5 in diagnosing IBD using a method of the invention is about 87.5%.

In a further embodiment, the ANCA, ASCA-IgA, and ASCA-IgG cut-off values "X," "Y," and "Z" can be independently selected, for example, such that the 10 negative predictive value in a patient population having an IBD disease prevalence of about 15% is at least about 95%.

The term "negative predictive value," as used herein, is synonymous with "NPV" and means the 15 probability that an individual diagnosed as not having IBD actually does not have the disease. Negative predictive value can be calculated as the number of true negatives divided by the sum of the true negatives and false negatives. Negative predictive value is determined 20 by the characteristics of the diagnostic method as well as the prevalence of the disease in the population analyzed. In a method of the invention, the ANCA, ASCA-IgA, and ASCA-IgG cut-off values can be selected such that the negative predictive value in a population 25 having an IBD disease prevalence is in the range of 80-99% and can be, for example, at least about 85%, at least about 90%, or at least about 95%. In particular, in a population having an IBD disease prevalence of 1 to 30 2%, the negative predictive value can be, for example, at least about 85%, 90%, 95%, 96%, 97%, 98% or 99%.

Predictive values, including negative and positive predictive values, are influenced by the

prevalence of the disease in the population analyzed. In the methods of the invention, the cut-off values X, Y and Z can be selected to produce a desired clinical parameter for a clinical population with a particular IBD disease prevalence. For example, cut-off values X, Y and Z can be selected for an IBD disease prevalence of about 10%, 12%, 15%, 18% or 20%, which can be seen, for example, in a gastroenterologist's office. Cut-off values X, Y, and Z also can be selected for an IBD disease prevalence of about 1%, 2%, 3%, 4%, 5%, 6%, 7% or 8%. An IBD disease prevalence of 1 to 2% is typical of the disease prevalence seen in a general doctor's office.

In a method of the invention, the ANCA, ASCA-IgA, and ASCA-IgG cut-off values "X," "Y," and "Z" can be selected such that, when the sensitivity of diagnosing an individual with IBD is at least about 70% and the specificity of diagnosing an individual with IBD is in the range of 30-60%, the negative predictive value in a population having an IBD disease prevalence of about 15% is at least about 90%. X, Y and Z can be selected such that, for example, the sensitivity is at least about 70%, the specificity is 30-60%, and the negative predictive value in a population having an IBD disease prevalence of about 15% is greater than 95%.

Furthermore, in a method of the invention, the ANCA, ASCA-IgA, and ASCA-IgG cut-off values "X," "Y," and "Z" can be selected such that, when the sensitivity of diagnosing an individual with IBD is at least about 90% and the specificity of diagnosing an individual with IBD is in the range of 20-60%, the negative predictive value in a population having an IBD disease prevalence of about 15% is at least about 90%, for example, at least about 95%.

In a method of the invention, the ANCA, ASCA-IgA, and ASCA-IgG cut-off values "X," "Y," and "Z" can be selected such that, when the sensitivity of diagnosing an individual with IBD is at least about 70% and the specificity of diagnosing an individual with IBD is in the range of 20-60%, the negative predictive value in a population having an IBD disease prevalence of about 1-2% is at least about 98%. The values X, Y and Z can be selected such that the sensitivity is at least about 90%, 10 the specificity of diagnosing an individual with IBD is 20-60%, and the negative predictive value in a population having an IBD disease prevalence of about 1-2% is greater than 98%. The negative predictive value in such a population can be, for example, greater than 99%.

15 In another embodiment, the ANCA, ASCA-IgA, and ASCA-IgG cut-off values "X," "Y," and "Z" can be independently selected such that, for example, the positive predictive value in a patient population having an IBD disease prevalence of about 15% is at least 20 about 5%.

The term "positive predictive value," as used herein, is synonymous with "PPV" and means the probability that an individual diagnosed as having IBD 25 actually has the disease. Positive predictive value can be calculated as the number of true positives divided by the sum of the true positives and false positives. Positive predictive value is determined by the characteristics of the diagnostic method as well as the 30 prevalence of the disease in the population analyzed. In a method of the invention, the ANCA, ASCA-IgA, and ASCA-IgG cut-off values can be selected such that the positive predictive value of the method in a population having an IBD disease prevalence of 15% is at least about

5%, and can be, for example, at least about 8%, 10%, 15%, 20%, 25%, 30% or 40%.

In a further embodiment of the invention, the
5 ANCA, ASCA-IgA, and ASCA-IgG cut-off values "X," "Y," and "Z" can be independently selected such that, for example, overall agreement in a patient population having an IBD disease prevalence of about 15% is at least about 45%.

As used herein, the term "overall agreement"
10 means the accuracy with which a method diagnoses a disease state. Overall agreement is calculated as the sum of the true positives and true negatives divided by the total number of sample results and is affected by the prevalence of IBD in the population analyzed. The ANCA, 15 ASCA-IgA, and ASCA-IgG cut-off values can be selected such that the overall agreement of a method of the invention in a patient population having an IBD disease prevalence of 15% is at least about 45%, and can be, for example, at least about 50%, 55% or 60%.

20 One skilled in the art can select an ANCA cut-off "X," an ASCA-IgA cut-off "Y," and an ASCA-IgG cut-off "Z" to achieve one or more clinically useful parameters, such as a desired sensitivity or specificity, or a desired negative predictive value, positive 25 predictive value or overall agreement for a patient population having a particular disease prevalence. Factorial Design Optimization or Design of Experiments methodology can be used, for example, to select an appropriate ANCA cut-off "X," an ASCA-IgA cut-off "Y," 30 and an ASCA-IgG cut-off "Z." As disclosed herein in Example II, optimization software (DOE Keep It Simple Statistically from Air Academy Associates (Colorado Springs, CO) was used in a central composite design

experiment to simultaneously vary the three ELISA cut-offs "X," "Y," and "Z." In particular, the base ANCA cut-off was varied from 0.5 to 1.5 times the base value of approximately 10 to 20 EU; the base ASCA-IgA cut-off 5 was varied from 10 EU to 30 EU; and the base ASCA-IgG cut-off was varied from 20 EU to 60 EU. By comparing the test results determined for the 851 individuals in the database (see Table 1) with the assigned "X," "Y," and "Z" cut-offs, each of the 851 samples were determined to 10 be a true positive, true negative, false positive or false negative, and the clinical parameters of sensitivity, specificity, negative predictive value, positive predictive value and overall agreement were determined. Using these results, an optimized set of 15 ANCA, ASCA-IgA and ASCA-IgG cut-off values were determined for each clinical parameter. Although the determination of the ANCA, ASCA-IgA and ASCA-IgG cut-off values "X," "Y," and "Z" is illustrated herein using the DOE KISS program, one skilled in the art understands that 20 other computer programs for identifying cooperative interactions among multiple variables and for performing simultaneous equation calculations also can be used. For example, ECHIP optimization software, available from ECHIP, Incorporated (Hockessin, DE), or Statgraphics 25 optimization software, available from STSC, Incorporated (Rockville, MD), also can be useful in determining the ANCA, ASCA-IgA and ASCA-IgG cut-off values in a method of the invention.

A variety of assay formats can be used to 30 determine ANCA, ASCA-IgA and ASCA-IgG levels in a sample. As described above, the methods of the present invention can be performed with whole cells, such as neutrophils for the determination of ANCA levels, or yeast for the determination of ASCA-IgA or ASCA-IgG levels; with

unpurified or partially purified cell extracts; or with purified proteins, protein fragments or peptides, which can be produced, for example, recombinantly, synthetically or using phage display technology.

5 Flow cytometry can be used to determine ANCA, ASCA-IgA and ASCA-IgG levels according to a method of the invention. Such flow cytometric assays, including bead based immunoassays, can be used to determine ANCA, ASCA-IgA and ASCA-IgG levels in the same manner as used
10 to detect serum antibodies to *Candida albicans* and serum antibodies to HIV proteins (see, for example, Bishop and Davis, J. Immunol. Methods 210:79-87 (1997); McHugh et al., J. Immunol. Methods 116:213 (1989); Scillian et al., Blood 73:2041 (1989), each of which is incorporated by
15 reference herein).

Phage display technology for expressing a recombinant antigen specific for ANCA or ASCA also can be used to determine the level of ANCA, ASCA-IgA or ASCA-IgG. Phage particles expressing the antigen
20 specific for ANCA, or an antigen specific for ASCA, can be anchored, if desired, to a multiwell plate using an antibody such as an antiphage monoclonal antibody (Felici et al., "Phage-Displayed Peptides as Tools for Characterization of Human Sera" in Abelson (Ed.), Methods
25 in Enzymol. 267, San Diego: Academic Press, Inc. (1996), which is incorporated by reference herein).

A variety of immunoassay formats including competitive and non-competitive immunoassay formats also are useful the methods of the invention (Self and Cook,
30 Curr. Opin. Biotechnol. 7:60-65 (1996), which is incorporated by reference). Immunoassays encompass capillary electrophoresis based immunoassays (CEIA) and

can be automated, if desired. Immunoassays also can be used in conjunction with laser induced fluorescence (see, for example, Schmalzing and Nashabeh, Electrophoresis 18:2184-93 (1997)); Bao, J. Chromatogr. B. Biomed. Sci. 5 699:463-80 (1997), each of which is incorporated herein by reference). Liposome immunoassays, such as flow-injection liposome immunoassays and liposome immunosensors, also can be used to determine ANCA, ASCA-IgA and ASCA-IgG levels according to a method of the 10 invention (Rongen et al., J. Immunol. Methods 204:105-133 (1997), which is incorporated by reference herein).

Immunoassays, such as enzyme-linked immunosorbent assays (ELISAs), can be particularly useful 15 in a method of the invention. A fixed neutrophil ELISA, for example, can be useful for determining whether a sample is positive for ANCA or for determining the ANCA level in a sample (see Example I). Similarly, an ELISA using yeast cell wall phosphopeptidomannan can be useful 20 for determining whether a sample is positive for ASCA-IgA or ASCA-IgG, or for determining the ASCA-IgA or ASCA-IgG levels in a sample. An enzyme such as horseradish peroxidase (HRP), alkaline phosphatase (AP), β -galactosidase or urease can be linked to a secondary 25 antibody selective for ANCA, or to a secondary antibody selective for ASCA for use in a method of the invention. A horseradish-peroxidase detection system can be used, for example, with the chromogenic substrate tetramethylbenzidine (TMB), which yields a soluble 30 product in the presence of hydrogen peroxide that is detectable at 450 nm. An alkaline phosphatase detection system can be used with the chromogenic substrate *p*-nitrophenyl phosphate, for example, which yields a soluble product readily detectable at 405 nm. Similarly, 35 a β -galactosidase detection system can be used with the

chromogenic substrate *o*-nitrophenyl- β -D-galactopyranoside (ONPG), which yields a soluble product detectable at 410 nm, or a urease detection system can be used with a substrate such as urea-bromocresol purple (Sigma

5 Immunochemicals, St. Louis, MO). A useful secondary antibody linked to an enzyme can be obtained from a number of commercial sources; goat F(ab'), anti-human IgG-alkaline phosphatase, for example, can be purchased from Jackson Immuno-Research (West Grove, PA).

10 A radioimmunoassay also can be useful for determining whether a sample is positive for ANCA, ASCA-IgA or ASCA-IgG, or for determining the level of ANCA, ASCA-IgA or ASCA-IgG in a sample. A radioimmunoassay using, for example, an iodine-125

15 labeled secondary antibody (Harlow and Lane, Antibodies A Laboratory Manual Cold Spring Harbor Laboratory: New York, 1988, which is incorporated herein by reference) is encompassed within the invention.

A secondary antibody labeled with a

20 chemiluminescent marker also can be useful in the methods of the invention. Such a chemiluminescent secondary antibody is convenient for sensitive, non-radioactive detection of ANCA, ASCA-IgA or ASCA-IgG and can be obtained commercially from various sources such as

25 Amersham Lifesciences, Inc. (Arlington Heights, IL).

In addition, a detectable reagent labeled with a fluorochrome can be useful in the methods of the invention for determining whether ANCA, ASCA-IgA or ASCA-IgG is present in a sample. Appropriate

30 fluorochromes include, for example, DAPI, fluorescein, Hoechst 33258, R-phycocyanin, B-phycoerythrin, R-phycoerythrin, rhodamine, Texas red or lissamine. A

particularly useful fluorochrome is fluorescein or rhodamine. Secondary antibodies linked to fluorochromes can be obtained commercially. For example, goat F(ab')₂ anti-human IgG-FITC is available from Tago Immunologicals 5 (Burlingame, CA).

A signal from the detectable reagent can be analyzed, for example, using a spectrophotometer to detect color from a chromogenic substrate; a radiation counter to detect radiation, such as a gamma counter for 10 detection of iodine-125; or a fluorometer to detect fluorescence in the presence of light of a certain wavelength. For detection of enzyme-linked reagents, a quantitative analysis of the amount of ANCA, ASCA-IgA or ASCA-IgG can be made using a spectrophotometer such as an 15 EMAX Microplate Reader (Molecular Devices, Menlo Park, CA) in accordance with the manufacturer's instructions. If desired, the assays of the invention can be automated or performed robotically, and the signal from multiple samples can be detected simultaneously.

20 Immunoassays using a secondary antibody selective for ANCA, or selective for ASCA-IgA, or selective for ASCA-IgG, are particularly useful in the methods of the invention. As used herein, the term "antibody" means a population of immunoglobulin 25 molecules, which can be polyclonal or monoclonal and of any isotype. As used herein, the term "antibody" encompasses an immunologically active fragment of an immunoglobulin molecule. Such an immunologically active fragment contains the heavy and light chain variable 30 regions, which make up the portion of the antibody molecule that specifically binds an antigen. For example, an immunologically active fragment of an immunoglobulin molecule known in the art as Fab, Fab' or

$F(ab')_2$ is included within the meaning of the term antibody.

The invention additionally provides a highly efficient method of analyzing multiple samples for IBD 5 by first assaying all samples for the presence or absence of ANCA; next assaying only ANCA-negative samples for the presence or absence of ASCA-IgA; and next assaying only ANCA-negative and ASCA-IgA-negative samples for the presence or absence of ASCA-IgG, where the presence of 10 pANCA, ASCA-IgA or ASCA-IgG in a sample is indicative of IBD and where the absence of ANCA, ASCA-IgA and ASCA-IgG is indicative of the absence of IBD. In such a method of the invention, the presence of ANCA, ASCA-IgA and ASCA-IgG can be conveniently determined, for example, 15 using an immunoassay.

The following examples are intended to illustrate but not limit the present invention.

EXAMPLE I
DETERMINATION OF PATIENT ANCA STATUS

20 This example describes analysis of patient ANCA, ASCA-IgA and ASCA-IgG levels using ELISA assays.

A. Fixed neutrophil ELISA for determining ANCA levels

A fixed neutrophil enzyme-linked immunosorbent 25 assay was used to detect ANCA as described in Saxon et al., *supra*, 1990. Briefly, microtiter plates were coated with 2.5×10^5 neutrophils per well from peripheral human blood purified by Ficoll-hypaque centrifugation and treated with 100% methanol for 10 minutes to fix the

cells. Cells were incubated with 0.25% bovine serum albumin (BSA) in phosphate-buffered saline to block nonspecific antibody binding for 60 minutes at room temperature in a humidified chamber. Next, control and 5 coded sera were added at a 1:100 dilution to the bovine serum/phosphate-buffered saline blocking buffer and incubated for 60 minutes at room temperature in a humidified chamber. Alkaline phosphatase-conjugated goat F(ab')₂ anti-human immunoglobulin G antibody (γ -chain 10 specific; Jackson Immunoresearch Labs, Inc., West Grove, PA) was added at a 1:1000 dilution to label neutrophil-bound antibody and incubated for 60 minutes at room temperature. A solution of *p*-nitrophenol phosphate substrate was added, and color development was allowed to 15 proceed until absorbance at 405 nm in the positive control wells was 0.8-1.0 optical density units greater than the absorbance in blank wells.

A panel of twenty verified negative control samples was used with a calibrator with a defined ELISA 20 Unit (EU) value. The base positive/negative cut-off for each ELISA run was defined as the optical density (OD) of the Calibrator minus the mean (OD) value for the panel of twenty negatives (plus 2 standard deviations) times the EU value of the Calibrator. The base cut-off value for 25 ANCA reactivity was therefore about 10 to 20 EU, with any patient sample having an average EU value greater than the base cut-off marked as ELISA positive for ANCA reactivity. Similarly, a patient sample having an average EU value is less than or equal to the base 30 cut-off is determined to be negative for ANCA reactivity.

B. Preparation of yeast cell wall mannan for ASCA ELISA assay

Yeast cell wall mannan was prepared as follows and as described in Faille et al., Eur. J. Clin. Microbiol. Infect. Dis. 11:438-446 (1992) and in Kocourek and Ballou et al., J. Bacteriol. 100:1175-1181 (1969), 5 each of which is incorporated herein by reference. A lyophilized pellet of yeast *Saccharomyces uvarum* was obtained from the American Type Culture Collection (#38926). Yeast were reconstituted in 10 ml 2X YT medium, prepared according to Sambrook et al., Molecular 10 Cloning Cold Spring Harbor Laboratory Press (1989), which is incorporated herein by reference. *S. uvarum* were grown for two to three days at 30°C. The terminal *S. uvarum* culture was inoculated on a 2X YT agar plate and subsequently grown for two to three days at 30°C. A 15 single colony was used to inoculate 500 ml 2X YT media, and grown for two to three days at 30°C. Fermentation media (pH 4.5) was prepared by adding 20 gm glucose, 2 gm bacto-yeast extract, 0.25 gm MgSO₄ and 2.0 ml 28% H₃PO₄ per liter distilled water. The 500 ml culture was used 20 to inoculate 50 liters of fermentation media, and the culture fermented for three to four days at 37°C.

S. uvarum mannan extract was prepared by adding 50 ml 0.02 M citrate buffer (5.88 gm/l sodium citrate; pH 7.0+/-0.1) to each 100 grams of cell paste. The 25 cell/citrate mixture was autoclaved at 125°C for ninety minutes and allowed to cool. After centrifuging at 5000 rpm for 10 minutes, the supernatant was removed and retained. The cells were then washed with 75 ml 0.02 M citrate buffer and the cell/citrate mixture again 30 autoclaved at 125°C for ninety minutes. The cell/citrate mixture was centrifuged at 5000 rpm for 10 minutes, and the supernatant retained.

In order to precipitate copper/mannan complexes, an equal volume of Fehling's Solution was added to the combined supernatants while stirring. The complete Fehling's solution was prepared by mixing 5 Fehling's Solution A with Fehling's Solution B in a 1:1 ratio just prior to use. The copper complexes were allowed to settle, and the liquid decanted gently from the precipitate. The copper/mannan precipitate complexes were then dissolved in 6-8 ml 3N HCl per 100 grams yeast 10 paste.

The resulting solution was poured with vigorous stirring into 100 ml of 8:1 methanol:acetic acid, and the precipitate allowed to settle for several hours. The 15 supernatant was decanted and discarded; then the wash procedure was repeated until the supernatant was colorless, approximately two to three times. The precipitate was collected on a scintered glass funnel, washed with methanol and air dried overnight. On some 20 occasions, the precipitate was collected by centrifugation at 5000 rpm for 10 minutes before washing with methanol and air drying overnight. The dried mannан powder was dissolved in distilled water, to a concentration of approximately 2 g/ml.

25 C. Preparation of *S. uvarum* mannан ELISA plates

S. uvarum cell mannан ELISA plates were saturated with antigen as follows. Purified *S. uvarum* mannан prepared as described above was diluted to a concentration of 100 μ g/ml with phosphate buffered 30 saline/0.2% sodium azide (PBS-N3). Using a multi-channel pipettor, 100 μ l of 100 μ g/ml *S. uvarum* mannан was added per well of a Costar 96-well hi-binding plate (catalogue number 3590; Costar Corp., Cambridge, MA). The antigen

was allowed to coat the plate at 4° C for a minimum of 12 hours. Each lot of plates was compared to a previous lot before use. Plates were stored at 2-8° C for up to one month.

5 D. ASCA ELISA analysis of patient sera

Patient sera were analyzed in duplicate for anti-IgG or anti-IgA reactivity. Microtiter plates saturated with antigen as described above were incubated with phosphate buffered saline/0.05% Tween-20 for 45 10 minutes at room temperature to inhibit nonspecific antibody binding. Patient sera were subsequently added at a dilution of 1:80 for ASCA-IgA and 1:800 for analysis of ASCA-IgG and incubated for 1 hour at room temperature. Wells were washed three times with PBS/0.05% Tween-20. 15 Then a 1:1000 dilution of alkaline phosphatase-conjugated goat anti-human IgA (Jackson Immunoresearch, West Grove, PA) or a 1:1000 dilution of alkaline phosphatase-conjugated goat anti-human IgG F(ab')₂ (Pierce, Rockford, IL) or was added, and the microtiter plates 20 incubated for 1 hour at room temperature. A solution of *p*-nitrophenol phosphate in diethanolamine substrate buffer was added, and color development allowed to proceed for 10 minutes. Absorbance at 405 nm was analyzed using an automated EMAX plate reader (Molecular 25 Devices, Sunnyvale, CA).

To determine the base cut-off value for ASCA-IgA and ASCA-IgG, single point calibrators having fixed EU values were used. OD values for patients samples were compared to the OD value for the Calibrators 30 and multiplied by the Calibrator assigned values. The base cut-off value for the ASCA-IgA ELISA was 20 EU. The base cut-off value for the ASCA-IgG was 40 EU.

EXAMPLE II**DETERMINATION OF OPTIMIZED CUT-OFFS FOR ANCA, ASCA-IgA
AND ASCA-IgG POSITIVITY**

This example demonstrates that particular ANCA,
5 ASCA-IgA and ASCA-IgG cut-off values can be selected to
yield a preferred clinical parameter for diagnosing IBD.

A. Database used in analysis

Only quantitative ELISA procedures were
performed and particular cut-off values for the results
10 of each ELISA assay used to determine whether the test
serum sample was positive or negative for markers of IBD.
In particular, no immunofluorescence assay procedures
were performed as part of these diagnostics.

The cut-off values for each of the three ELISA
15 components of the assay were determined using a database
consisting of serology data from 851 individuals
(Table 1). The presence or absence of inflammatory bowel
disease was made for all IBD patients by colonoscopic,
radiologic, and/or histologic methods at Cedars Sinai
20 Medical Center (Los Angeles, California). Serum from 300
asymptomatic non-disease controls also was tested,
although, for ethical reasons, colonoscopy was not
performed on these individuals.

Table 1
ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
5	1	NON IBD	NON IBD	8.624	13.374	5.356	15.516
	2	NON IBD	NON IBD	6.606	13.374	1.379	10.28
	3	NON IBD	NON IBD	6.056	13.374	3.995	5.874
	4	NON IBD	NON IBD	9.602	13.374	1.697	12.77
	5	NON IBD	NON IBD	6.85	13.374	1.626	6.449
10	6	NON IBD	NON IBD	7.462	13.374	1.997	12.898
	7	NON IBD	NON IBD	7.278	13.374	2.669	8.684
	8	NON IBD	NON IBD	15.229	13.374	2.881	11.429
	9	NON IBD	NON IBD	11.743	13.374	1.591	17.431
	10	NON IBD	NON IBD	13.7	13.374	0.389	4.533
15	11	NON IBD	NON IBD	12.783	13.374	2.492	23.434
	12	NON IBD	NON IBD	11.07	13.374	3.853	6.96
	13	NON IBD	NON IBD	9.297	13.374	11.754	10.599
	14	NON IBD	NON IBD	7.951	13.374	0.548	4.533
	15	NON IBD	NON IBD	9.833	11.989	0.716	6.065
20	16	NON IBD	NON IBD	8.135	13.374	0.813	5.938
	17	NON IBD	NON IBD	7.829	13.374	12.39	7.471
	18	NON IBD	NON IBD	11.988	13.374	2.863	26.371
	19	NON IBD	NON IBD	5.015	13.374	1.891	5.938
	20	NON IBD	NON IBD	9.358	13.374	1.98	4.278
25	21	NON IBD	NON IBD	8.073	13.374	1.573	5.747
	22	NON IBD	NON IBD	9.419	13.374	2.492	11.94
	23	NON IBD	NON IBD	5.015	13.374	1.962	6.641
	24	NON IBD	NON IBD	7.278	13.374	1.502	13.537
	25	NON IBD	NON IBD	9.419	13.374	3.252	8.875
30	26	NON IBD	NON IBD	9.174	13.374	0.442	4.883
	27	NON IBD	NON IBD	19.388	13.374	5.285	7.854
	28	NON IBD	NON IBD	12.355	13.374	2.563	5.108
	29	NON IBD	NON IBD	6.544	13.374	5.638	5.172
	30	NON IBD	NON IBD	7.278	13.374	13.504	15.58
35	31	NON IBD	NON IBD	7.645	13.374	4.277	27.137
	32	NON IBD	NON IBD	8.624	13.374	2.722	4.661

Table 1
ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
	33	NON IBD	NON IBD	20.183	13.374	3.411	6.002
	34	NON IBD	NON IBD	14.434	13.374	15.006	19.794
5	35	NON IBD	NON IBD	16.566	20.956	6.345	12.643
	36	NON IBD	NON IBD	17.9	20.956	13.398	17.559
	37	NON IBD	NON IBD	10.801	20.956	2.474	5.683
	38	NON IBD	NON IBD	23.726	20.956	22.642	37.034
	39	NON IBD	NON IBD	16.323	20.956	1.803	18.198
	40	NON IBD	NON IBD	19.114	20.956	4.313	11.429
	41	NON IBD	NON IBD	22.33	20.956	3.888	5.108
10	42	NON IBD	NON IBD	6.371	20.956	2.404	2.554
	43	NON IBD	NON IBD	17.597	20.956	13.804	19.475
	44	NON IBD	NON IBD	14.745	20.956	1.326	2.937
	45	NON IBD	NON IBD	7.16	20.956	1.827	4.373
	46	NON IBD	NON IBD	13.896	20.956	1.292	2.345
15	47	NON IBD	NON IBD	7.585	20.956	1.846	6.782
	48	NON IBD	NON IBD	10.437	20.956	0.941	5.134
	49	NON IBD	NON IBD	7.282	20.956	1.034	21.296
	50	NON IBD	NON IBD	13.774	20.956	1.569	7.606
	51	NON IBD	NON IBD	17.718	20.956	2.215	6.021
20	52	NON IBD	NON IBD	11.408	20.956	0.646	2.535
	53	NON IBD	NON IBD	7.949	20.956	2.363	9.571
	54	NON IBD	NON IBD	14.684	20.956	1.218	5.704
	55	NON IBD	NON IBD	14.867	20.956	3.784	7.986
	56	NON IBD	NON IBD	11.529	20.956	1.2	10.775
25	57	NON IBD	NON IBD	21.177	20.956	2.196	8.43
	58	NON IBD	NON IBD	9.527	20.956	2.639	9.697
	59	NON IBD	NON IBD	12.985	20.956	2.16	5.894
	60	NON IBD	NON IBD	11.59	20.956	2.436	22.881
	61	NON IBD	NON IBD	10.255	20.956	0.554	2.028
30	62	NON IBD	NON IBD	13.592	20.956	1.218	5.958
	63	NON IBD	NON IBD	13.532	20.956	1.347	15.211
	64	NON IBD	NON IBD	12.500	20.956	11.228	98.739

Table 1
ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
5	65	NON IBD	NON IBD	12.925	20.956	2.170	12.131
	66	NON IBD	NON IBD	12.318	20.956	3.780	12.907
	67	NON IBD	NON IBD	16.262	20.956	7.717	18.478
	68	NON IBD	NON IBD	8.897	21.656	1.767	3.667
	69	NON IBD	NON IBD	7.374	21.656	1.767	6.7
	70	NON IBD	NON IBD	17.550	21.656	0.895	33.501
	71	NON IBD	NON IBD	20.963	21.656	6.554	14.247
	72	NON IBD	NON IBD	36.868	21.656	2.774	7.264
	73	NON IBD	NON IBD	15.113	21.656	1.566	12.342
	74	NON IBD	NON IBD	12.371	21.656	12.749	9.521
10	75	NON IBD	NON IBD	13.163	21.656	2.505	4.373
	76	NON IBD	NON IBD	20.353	21.656	2.505	8.745
	77	NON IBD	NON IBD	28.032	21.656	4.317	28.211
	78	NON IBD	NON IBD	14.869	21.656	1.253	4.866
	79	NON IBD	NON IBD	15.174	21.656	4.719	10.297
	80	NON IBD	NON IBD	18.952	21.656	5.681	32.937
	81	NON IBD	NON IBD	25.960	21.656	36.347	43.868
	82	NON IBD	NON IBD	18.342	21.656	2.438	11.426
	83	NON IBD	NON IBD	21.511	21.656	1.096	5.36
	84	NON IBD	NON IBD	17.002	21.656	1.856	6.841
20	85	NON IBD	NON IBD	18.282	21.656	4.004	7.758
	86	NON IBD	NON IBD	8.775	21.656	2.572	2.116
	87	NON IBD	NON IBD	12.066	21.656	4.742	8.534
	88	NON IBD	NON IBD	15.844	21.656	4.563	29.974
	89	NON IBD	NON IBD	15.722	21.656	1.230	2.892
	90	NON IBD	NON IBD	13.528	21.656	1.163	4.373
	91	NON IBD	NON IBD	19.988	21.656	6.129	9.169
	92	NON IBD	NON IBD	17.002	21.656	4.317	25.672
	93	NON IBD	NON IBD	18.647	21.656	0.962	6.982
	94	NON IBD	NON IBD	17.733	21.656	15.545	33.994
30	95	NON IBD	NON IBD	16.819	21.656	9.327	16.715
	96	NON IBD	NON IBD	17.550	21.656	7.269	18.196

Table 1
ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
5	97	NON IBD	NON IBD	13.633	21.656	3.422	9.733
	98	NON IBD	NON IBD	7.861	21.656	10.244	7.123
	99	NON IBD	NON IBD	14.747	21.656	2.930	13.048
	100	NON IBD	NON IBD	15.661	21.656	3.623	15.869
	101	NON IBD	NON IBD	15.697	11.989	3.646	13.4
	102	NON IBD	NON IBD	11.231	11.989	2.214	10.156
	103	CD	IBD	10.311	17.023	11.264	14.32
	104	CD	IBD	49.604	17.023	4.613	45.98
	105	107	CD	12.919	17.023	130.938	62.82
	106	108	CD	21.242	17.023	115.841	85.92
10	107	110	CD	35.031	17.023	61.667	57.82
	108	111	UC	6.708	17.023	31.708	36.87
	109	113	UC	16.149	17.023	18.369	12.54
	110	114	CD	19.565	17.023	22.501	31.03
	111	115	CD	23.168	17.023	45.229	33.71
	112	116	CD	16.335	17.023	10.974	7.46
	113	118	UC	24.161	17.023	20.816	49.51
	114	119	UC	74.596	17.023	8.419	13.82
	115	122	UC	150.215	17.023	4.178	7.80
	116	126	CD	17.019	17.023	128.111	84.87
20	117	127	CD	28.509	17.023	42.546	40.00
	118	134	CD	30.932	17.023	9.071	8.27
	119	136	UC	51.977	17.023	67.842	16.81
	120	137	UC	98.417	17.023	4.558	13.78
	121	138	CONTROL	14.845	17.023	2.492	13.88
	122	139	CD	9.503	17.023	5.682	8.57
	123	140	CD	49.683	17.023	18.840	10.66
	124	142	UC	118.275	17.023	1.912	4.30
	125	143	CD	22.919	17.023	4.993	11.66
	126	144	CD	18.820	17.023	55.270	27.43
30	127	145	CD	29.130	17.023	137.028	118.39
	128	146	CONTROL	38.199	17.023	18.677	19.53

Table 1

ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
5	129	147	UC	IBD 58.306	17.023	2.347	5.71
	130	148	CD	IBD 44.472	17.023	41.658	25.58
	131	149	CD	IBD 65.189	17.023	41.260	14.55
	132	157	CD	IBD 17.888	17.023	27.431	24.61
	133	158	CD	IBD 54.836	17.023	61.831	24.91
	134	160	UC	IBD 162.181	17.023	8.292	9.82
	135	166	CD	IBD 27.578	17.023	36.964	22.86
	136	168	CD	IBD 21.615	17.023	134.527	160.93
	137	169	UC	IBD 88.000	17.023	9.434	8.15
	138	170	CD	IBD 32.298	17.023	71.201	48.56
10	139	174	UC	IBD 56.072	17.023	10.720	15.04
	140	175	CD	IBD 43.416	17.023	2.691	14.38
	141	176	NON IBD	NON IBD 14.472	17.023	6.588	5.68
	142	178	CD	IBD 43.054	17.023	80.807	64.06
	143	201	CD	IBD 16.211	17.023	31.980	35.14
	144	203	CD	IBD 18.758	17.023	73.394	35.85
	145	204	CD	IBD 58.400	17.023	57.010	112.83
	146	206	CD	IBD 10.870	17.023	47.386	34.81
	147	207	CD	IBD 11.304	17.023	95.421	60.64
	148	213	CD	IBD 34.721	17.023	39.549	15.55
15	149	214	CD	IBD 20.311	17.023	27.289	25.37
	150	216	CD	IBD 43.272	17.023	57.880	33.85
	151	218	CD	IBD 21.677	17.023	23.242	36.56
	152	221	NON IBD	NON IBD 15.776	17.023	22.296	10.74
	153	223	CONTROL	NON IBD 8.025	15.673	16.938	9.44
	154	224	CD	IBD 14.502	15.673	27.256	52.74
	155	229	CD	IBD 13.474	15.673	8.129	14.92
	156	231	CD	IBD 50.254	15.673	97.014	69.24
	157	234	CD	IBD 46.690	15.673	45.405	36.68
	158	236	UC	IBD 32.568	15.673	7.150	41.93
30	159	237	UC	IBD 70.695	15.673	7.780	8.56
	160	238	CONTROL	NON IBD 11.239	15.673	2.820	10.32

Table 1

ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)	
161	247	UC	IBD	24.109	15.673	25.747	32.92	
162	248	CD	IBD	19.517	15.673	14.615	21.30	
163	249	CD	IBD	5.740	15.673	3.368	5.89	
164	253	CD	IBD	30.453	15.673	7.697	17.54	
5	165	254	UC	70.028	15.673	4.745	15.50	
166	258	NON IBD	NON IBD	11.904	15.673	4.197	10.59	
167	260	CD	IBD	59.238	15.673	7.100	44.96	
10	168	261	NON IBD	NON IBD	41.390	15.673	3.019	9.98
169	262	CD	IBD	7.855	15.673	2.273	11.56	
170	265	UC	IBD	73.131	15.673	4.214	10.80	
171	267	CD	IBD	20.181	15.673	72.512	23.45	
172	274	UC	IBD	139.985	15.673	14.267	11.47	
173	275	CD	IBD	25.921	15.673	10.186	11.62	
15	174	276	UC	93.582	15.673	11.347	18.33	
175	278	CD	IBD	94.005	15.673	86.447	97.21	
176	314	UC	IBD	15.529	15.673	8.245	15.56	
177	321	UC	IBD	157.281	15.673	11.032	16.35	
178	325	NON IBD	NON IBD	13.716	15.673	10.687	27.15	
20	179	329	CD	8.097	15.673	3.878	7.68	
180	334	IBS	NON IBD	54.583	15.673	3.557	26.79	
181	339	UC	IBD	55.589	15.673	3.846	9.28	
182	352	CD	IBD	24.592	15.673	124.983	129.22	
183	362	UC	IBD	17.100	15.673	1.698	5.49	
25	184	374	NON IBD	NON IBD	9.305	15.673	6.885	13.80
185	376	CD	IBD	13.172	15.673	119.436	18.33	
186	377	NON IBD	NON IBD	12.508	15.673	18.331	8.83	
187	384	UC	IBD	65.257	15.673	4.529	6.64	
188	403	UC	IBD	83.950	15.673	18.613	36.49	
189	405	UC	IBD	89.737	15.673	13.869	27.63	
30	190	406	CONTROL	NON IBD	19.795	15.673	5.889	12.40
191	407	CD	IBD	6.949	15.673	4.960	29.41	
192	409	CD	IBD	114.898	15.673	18.547	26.60	

Table 1
ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)	
193	413	UC	IBD	24.900	15.673	2.107	9.60	
194	413	UC	IBD	24.894	15.673	1.955	9.6	
195	414	CD	IBD	54.475	15.673	67.817	102.23	
196	416	CONTROL	NON IBD	15.693	15.673	6.437	31.19	
5	197	417	UC	IBD	133.897	15.673	4.147	19.22
198	419	CD	IBD	4.411	15.673	27.090	79.19	
199	420	CD	IBD	23.021	15.673	5.410	32.59	
200	421	CD	IBD	65.500	15.673	43.334	30.13	
10	201	421	CD	IBD	65.509	15.673	23.073	30.13
202	422	CONTROL	NON IBD	22.425	15.673	8.714	29.56	
203	423	UC	IBD	37.523	15.673	7.942	10.85	
204	424	CD	IBD	18.429	15.673	33.448	32.74	
15	205	425	IBS	NON IBD	6.100	15.673	0.813	3.35
206	425	NON IBD	NON IBD	6.103	15.673	0.625	3.35	
207	426	NON IBD	NON IBD	14.743	15.673	4.150	4.90	
208	428	CD	IBD	57.780	15.673	7.583	9.45	
209	429	CD	IBD	36.469	15.673	6.894	9.18	
210	434	NON IBD	NON IBD	16.073	15.673	-1.351	16.11	
20	211	437	IBS	NON IBD	16.435	15.673	5.763	17.40
212	438	CD	IBD	64.824	15.673	60.141	36.22	
213	443	IBS	NON IBD	10.808	15.673	-0.262	6.12	
214	444	CD	IBD	26.326	15.673	27.134	42.89	
215	449	CONTROL	NON IBD	28.266	15.673	16.531	36.49	
25	216	450	UC	IBD	29.598	15.673	3.157	18.20
217	451	IBS	NON IBD	7.238	15.673	-2.344	5.81	
218	453	UC/PSC	IBD	58.404	15.673	7.555	12.67	
219	455	UC	IBD	65.354	15.673	11.375	16.34	
220	458	CONTROL	NON IBD	38.163	15.673	3.819	8.54	
221	461	UC	IBD	50.694	15.673	28.292	14.90	
30	222	464	CD	IBD	30.094	15.673	100.041	126.70
223	502	CD	IBD	6.247	15.673	8.727	26.30	
224	504	NON IBD	NON IBD	14.080	15.673	0.345	16.80	

Table 1
ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT	ASCA-IgG RESULT (EU)
	225	505	NON IBD	NON IBD 9.321	15.673	5.418	20.21
	226	506	CONTROL	NON IBD 23.451	15.673	12.643	36.35
5	227	509	CD	IBD 64.225	15.673	10.258	25.51
	228	515	CONTROL	NON IBD 17.744	15.673	8.934	23.81
	229	531	CD	IBD 198.079	15.673	4.829	20.58
	230	534	CONTROL	NON IBD 30.049	15.673	12.241	43.53
10	231	536	IBS	NON IBD 67.281	54.120	14.554	34.46
	232	537	NON IBD	NON IBD 22.607	24.358	1.988	21.49
	233	538	CD	IBD 65.898	54.120	98.850	174.86
15	234	539	CD	IBD 14.477	24.358	3.463	22.89
	235	542	CONTROL	NON IBD 11.057	15.673	4.234	30.99
	236	543	UC	IBD 69.278	54.120	7.521	21.74
	237	544	CD	IBD 15.915	24.358	27.458	57.35
20	238	545	NON IBD	NON IBD 18.245	24.358	53.402	90.17
	239	547	IBS	NON IBD 9.817	24.358	13.080	34.58
	240	551	IBS	NON IBD 12.643	24.358	4.220	36.53
	241	552	CD	IBD 71.812	54.120	16.313	43.47
	242	553	CD	IBD 30.788	24.358	11.335	69.35
25	243	554	CD	IBD 17.501	24.358	80.752	97.96
	244	557	NON IBD	NON IBD 11.701	24.358	5.248	24.11
	245	559	UC	IBD 90.564	69.938	5.140	28.25
	246	560	IBS	NON IBD 17.253	24.358	5.343	61.57
	247	561	UC	IBD 17.005	24.358	20.506	22.53
30	248	562	UC	IBD 23.688	20.189	38.929	26.18
	249	563	CD	IBD 26.338	20.189	3.076	47.37
	250	566	CONTROL	NON IBD 18.601	20.189	5.613	32.88
	251	567	UC	IBD 79.012	69.938	6.154	11.29
	252	569	CD	IBD 19.873	20.189	-0.054	26.60
	253	570	UC	IBD 111.640	20.189	14.351	19.49
	254	574	IBS	NON IBD 20.138	20.189	2.448	13.77
	255	577	IBS	NON IBD 13.620	20.189	0.460	2.68
	256	578	UC	IBD 21.516	20.189	0.852	4.78

Table 1

ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
257	579	UC	IBD	93.827	69.938	-0.257	5.64
258	580	CD	IBD	18.283	20.189	23.874	16.22
259	581	CD	IBD	18.177	20.189	61.923	48.26
260	601	CD	IBD	20.880	20.189	1.772	6.20
261	603	UC	IBD	78.571	69.938	4.153	6.20
262	604	UC	IBD	189.947	69.938	7.291	27.68
263	605	UC	IBD	37.149	20.189	3.896	9.00
264	608	UC	IBD	76.984	69.934	29.533	23.72
265	609	UC/PSC	IBD	60.934	69.934	22.143	24.90
266	610	UC	IBD	67.356	20.189	4.634	3.63
267	613	UC	IBD	50.026	20.189	5.018	22.93
268	616	UC	IBD	86.507	69.938	5.519	12.9
269	622	UC	IBD	50.609	20.189	9.327	13.93
270	623	UC	IBD	117.724	69.938	6.198	8.48
271	625	CD	IBD	13.937	20.189	27.597	39.58
272	627	NON IBD	NON IBD	13.620	20.189	2.420	6.24
273	628	CD	IBD	77.865	69.938	131.730	225.41
274	629	UC/PSC	IBD	27.663	20.189	2.656	4.62
275	631	IBS	NON IBD	14.467	20.189	5.224	10.54
276	632	UC	IBD	86.963	20.189	6.523	12.43
277	633	NON IBD	NON IBD	18.972	20.189	1.623	3.47
278	634	IBS	NON IBD	17.064	20.189	3.040	6.55
279	637	UC	IBD	154.637	20.189	4.162	3.51
280	639	UC	IBD	85.957	20.189	6.612	18
281	647	NON IBD	NON IBD	14.891	20.189	2.066	6.08
282	648	UC	IBD	63.328	20.189	4.309	110.18
283	650	CD	IBD	38.951	20.189	157.231	92.74
284	651	UC	IBD	59.088	20.189	47.816	34.93
285	660	UC	IBD	85.841	16.705	5.106	17.09
286	661	NON IBD	NON IBD	12.049	16.705	2.273	7.56
287	663	CD	IBD	9.606	16.705	89.138	61.33
288	667	UC	IBD	27.762	16.705	4.723	10.12

Table 1

ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
289	668	UC	IBD	9.328	16.705	2.509	8.84
290	669	CD	IBD	14.881	16.705	1.860	11.31
291	672	UC	IBD	116.380	16.705	4.634	19.16
292	678	CD	IBD	9.717	16.705	2.037	17.43
293	679	CD	IBD	78.956	16.705	0.974	9.78
294	681	UC	IBD	8.329	16.705	1.771	16.25
295	702	CD	IBD	6.441	16.705	1.033	2.57
296	703	CD	IBD	18.656	16.705	87.426	81.04
297	704	UC	IBD	33.981	16.705	11.959	24.79
298	705	CD	IBD	35.702	16.705	16.102	23.65
299	706	UC	IBD	10.161	16.705	122.505	87.7
300	707	UC	IBD	12.993	16.705	4.175	18.02
301	709	CD	IBD	42.754	16.705	60.923	71.46
302	711	IBS	NON IBD	17.990	16.705	7.094	15.51
303	712	CD	IBD	19.267	16.705	38.449	38.57
304	714	CD	IBD	74.014	16.705	1.820	3.85
305	716	UC	IBD	127.263	16.705	5.618	12
306	717	CD	IBD	11.105	16.705	62.398	37.83
307	718	UC	IBD	34.703	16.705	5.085	33.09
308	719	CD	IBD	23.320	16.705	5.304	12.89
309	720	CD	IBD	38.701	16.705	165.851	115.85
310	721	UC	IBD	101.444	16.705	1.507	4.64
311	725	IBS	NON IBD	16.687	23.307	3.296	8.99
312	727	CD	IBD	16.872	23.307	2.103	22.75
313	728	CD	IBD	36.761	23.307	8.600	8.29
314	729	UC	IBD	94.828	23.307	2.103	7.48
315	730	CD	IBD	15.517	23.307	12.084	10.52
316	732	UC	IBD	18.350	23.307	1.507	7.33
317	733	CD	IBD	19.150	23.307	71.124	31.03
318	735	UC	IBD	41.010	23.307	3.453	11.44
319	736	CD	IBD	14.224	23.307	142.655	72.03
320	737	NON IBD	NON IBD	5.788	23.307	-0.628	9.91

Table 1

ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
5	321	738	NON IBD	NON IBD 10.776	23.307	1.255	6.48
5	322	739	UC	IBD 14.840	23.307	6.403	5.87
5	323	740	NON IBD	NON IBD 14.286	23.307	5.116	18.83
5	324	741	CD	IBD 57.389	23.307	113.308	205.71
10	325	742	UC	IBD 33.744	23.307	4.018	7.41
10	326	743	UC	IBD 26.478	23.307	2.291	8.67
10	327	744	NON IBD	NON IBD 11.576	23.307	0.220	10.87
10	328	745	UC	IBD 36.392	23.307	-0.157	2.48
10	329	746	IBS	NON IBD 19.150	23.307	3.013	4.6
10	330	747	NON IBD	NON IBD 15.702	23.307	0.471	9.29
15	331	748	CD	IBD 18.350	23.307	0.471	10.68
15	332	752	UC	IBD 86.084	23.307	9.856	5.17
15	333	753	CD	IBD 19.212	23.307	71.030	31.03
15	334	754	UC	IBD 23.584	23.307	3.986	2.52
15	335	755	CD	IBD 57.143	23.307	18.613	41.49
15	336	756	CD	IBD 19.951	23.307	210.264	157.55
15	337	758	CD	IBD 11.761	23.307	53.233	64.84
20	338	760	UC	IBD 35.160	23.307	17.420	33.03
20	339	761	CONTROL	NON IBD 26.152	20.930	1.682	7.67
20	340	762	UC	IBD 47.260	20.930	2.548	7.98
20	341	763	CD	IBD 29.016	20.930	40.987	10.6
20	342	764	CONTROL	NON IBD 24.159	20.930	2.035	6.94
20	343	767	IBS	NON IBD 17.995	20.930	38.776	27.37
25	344	768	NON IBD	NON IBD 17.435	20.930	14.245	33.41
25	345	769	CD	IBD 19.614	20.930	1.266	2.37
25	346	770	UC	IBD 52.179	20.930	2.964	5.22
25	347	771	UC	IBD 52.428	20.930	11.489	21.22
25	348	773	UC	IBD 38.854	20.930	26.678	31.49
30	349	802	NON IBD	NON IBD 18.182	20.930	7.435	15.91
30	350	803	CD	IBD 42.403	20.930	82.583	89.88
30	351	804	NON IBD	NON IBD 16.438	20.930	1.234	8.52
30	352	805	CD	IBD 63.263	20.930	34.321	75.73

Table 1
 ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person
 database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
5	353	806	CD	IBD 20.735	20.930	46.803	65.15
	354	809	UC	IBD 81.133	20.930	1.009	4.48
	355	810	CD	IBD 25.716	20.930	104.054	62.92
	356	811	CD	IBD 15.567	20.930	76.334	64.38
	357	813	UC	IBD 51.681	20.930	0.641	8.23
	358	814	CD	IBD 18.182	20.930	26.598	68.68
	359	816	CD	IBD 24.844	20.930	10.880	27.28
	360	817	UC	IBD 33.686	20.930	1.394	9.78
	361	820	UC	IBD 31.756	20.930	5.704	13.15
	362	823	UC	IBD 23.848	20.930	1.058	4.5
10	363	828	CD	IBD 19.676	20.930	35.347	105.74
	364	830	UC	IBD 24.222	20.930	1.859	9.63
	365	831	UC	IBD 72.727	20.930	4.278	13.47
	366	832	UC	IBD 85.367	20.930	10.399	8.05
	367	833	UC	IBD 18.804	20.930	2.115	19.43
	368	834	UC	IBD 26.588	20.930	4.198	14.73
	369	835	CD	IBD 13.138	20.930	5.405	55.37
	370	836	CD	IBD 16.376	20.930	39.533	23.98
	371	837	NON IBD	NON IBD 6.351	20.930	12.597	36.86
	372	839	CD	IBD 16.252	20.930	118.140	145.7
15	373	841	UC	IBD 15.567	20.930	1.619	7.7
	374	862	CD	IBD 20.152	15.673	93.814	111.16
	375	863	CD	IBD 38.431	15.673	2.410	34.92
	376	874	CD	IBD 9.496	15.673	29.593	62.83
	377	875	CD	IBD 50.111	15.673	12.453	12.57
	378	905	CD	IBD 34.909	15.673	5.316	5.59
	379	906	CD	IBD 34.641	15.673	66.149	56.76
	380	914	CD	IBD 33.972	15.673	12.199	14.98
	381	929	CD	IBD 9.184	15.673	32.325	19.78
	382	930	UC	IBD 33.839	15.673	5.651	16.69
20	383	939	UC	IBD 114.044	15.673	2.825	7.33
	384	940	IBS	NON IBD 2.987	15.673	3.656	4.45

Table 1

ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
385	942	CD	IBD	13.286	15.673	81.427	51.87
386	943	CD	IBD	4.547	15.673	3.990	5.91
387	945	CD	IBD	12.158	11.390	6.856	21.79
388	947	CD	IBD	5.410	11.390	26.312	69.17
389	948	UC	IBD	20.790	11.390	6.387	8.38
390	949	UC	IBD	132.219	11.390	3.508	9.13
391	950	CD	IBD	9.666	11.390	3.575	7.12
392	951	UC	IBD	45.350	11.390	13.484	33.78
393	954	CD	IBD	11.307	11.390	39.743	38.29
394	955	UC	IBD	23.647	11.390	1.366	8.29
395	957	UC	IBD	18.541	11.390	4.874	9.17
396	959	IBS	NON IBD	10.274	11.390	4.379	4.36
397	961	UC	IBD	11.611	11.390	0.951	17.45
398	962	UC	IBD	12.584	11.390	0.589	7.12
399	963	UC	IBD	19.939	11.390	2.906	30.43
400	964	UC	IBD	38.116	11.390	3.696	11.70
401	965	IBS	NON IBD	6.505	11.390	1.861	2.82
402	966	IBS	NON IBD	6.505	11.390	11.061	10.45
403	967	CD	IBD	7.234	11.390	27.009	102.39
404	968	NON IBD	NON IBD	18.541	11.390	4.111	9.36
405	969	IBS	NON IBD	12.462	11.390	6.709	21.60
406	970	CD	IBD	29.119	11.390	76.995	40.16
407	971	CD	IBD	19.453	11.390	91.885	74.55
408	972	CD	IBD	11.672	11.390	43.198	104.83
409	973	UC	IBD	29.240	11.390	2.384	9.65
410	974	UC	IBD	21.033	11.390	6.909	9.93
411	979	CD	IBD	26.261	11.390	88.431	166.66
412	1003	NON IBD	NON IBD	4.216	10.493	3.878	13.01
413	1004	CD	IBD	3.972	10.493	7.574	18.13
414	1005	CD	IBD	27.767	10.493	12.185	44.66
415	1007	CD	IBD	11.755	10.493	0.763	9.02
416	1008	UC	IBD	38.346	10.493	25.683	13.29

Table 1
ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person
database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
	417	1009	UC	IBD 5.310	10.493	4.031	11.30
	418	1011	NON IBD	NON IBD 5.918	10.493	3.791	4.64
5	419	1014	CD	IBD 33.847	10.493	3.901	7.71
	420	1017	NON IBD	NON IBD 2.351	10.493	0.069	2.87
	421	1020	UC	IBD 10.215	10.493	3.846	17.73
	422	1022	CONTROL	NON IBD 6.972	10.493	3.878	16.03
	423	1025	UC	IBD 40.940	10.493	2.541	34.58
	424	1026	CONTROL	NON IBD 11.715	10.493	7.574	59.29
10	425	1029	NON IBD	NON IBD 4.580	10.493	6.991	11.24
	426	1031	CONTROL	NON IBD 5.432	10.493	14.558	7.66
	427	1032	NON IBD	NON IBD 1.500	10.493	3.063	12.84
	428	1034	WEGENER'S	NON IBD 76.976	10.493	9.463	25.99
	429	1035	CONTROL	NON IBD 9.972	10.493	15.726	32.48
	430	1040	UC	IBD 28.334	10.493	3.118	15.00
15	431	1042	UC	IBD 70.409	10.493	4.477	11.70
	432	1043	UC	IBD 16.214	10.493	7.595	10.05
	433	1044	UC	IBD 36.927	10.493	1.538	8.51
	434	1045	UC	IBD 20.916	10.493	6.139	14.55
	435	1046	UC	IBD 31.658	10.493	3.390	8.51
20	436	1047	UC	IBD 121.078	10.493	41.656	22.63
	437	1048	UC	IBD 46.500	10.493	7.142	12.84
	438	1048	UC	IBD 46.453	10.493	3.970	12.84
	439	1049	UC	IBD 110.661	10.493	38.580	25.70
	440	1050	UC	IBD 21.970	10.493	3.063	3.50
25	441	1051	UC	IBD 44.751	10.493	1.058	18.46
	442	1052	CD	IBD 29.179	11.390	7.183	39.51
	443	1054	UC	IBD 9.688	10.493	4.162	19.40
	444	1055	UC	IBD 7.621	10.493	43.662	35.22
	445	1056	UC	IBD 61.411	10.493	21.796	79.56
30	446	1057	CONTROL	NON IBD 8.188	10.493	1.799	12.03
	447	1058	UC	IBD 16.052	10.493	12.842	66.21
	448	1059	UC	IBD 10.230	12.074	16.852	19.23

Table 1
ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
449	1061	UC	IBD	29.967	12.074	5.439	48.63
450	1062	UC	IBD	6.820	12.074	0.247	5.33
451	1063	UC	IBD	3.148	12.074	3.214	8.46
452	1064	UC	IBD	10.303	12.074	2.362	10.49
453	1066	UC	IBD	40.852	12.074	8.680	14.01
454	1067	UC	IBD	11.148	12.074	1.112	3.96
455	1068	UC	IBD	11.803	12.074	4.299	6.26
456	1069	UC	IBD	15.279	12.074	1.387	2.47
457	1070	UC	IBD	5.508	12.074	1.195	7.53
458	1071	UC	IBD	2.885	12.074	6.167	10.71
459	1072	UC	IBD	70.230	12.074	28.554	18.46
460	1073	UC	IBD	25.508	12.074	3.379	9.56
461	1074	UC	IBD	48.131	12.074	8.721	12.97
462	1075	CONTROL	NON IBD	6.557	12.074	5.700	13.02
463	1076	CONTROL	NON IBD	3.410	12.074	0.481	6.87
464	1077	UC	IBD	29.605	11.390	7.760	8.08
465	1080	CONTROL	NON IBD	3.607	12.074	2.884	15.44
466	1081	UC	IBD	45.115	12.074	2.474	9.45
467	1106	UC	IBD	13.435	11.390	7.664	6.92
468	1107	UC	IBD	45.410	11.390	10.507	20.88
469	1109	UC	IBD	19.149	11.390	10.739	19.07
470	1110	UC	IBD	137.812	11.390	18.189	12.55
471	1111	UC	IBD	20.182	11.390	8.038	49.95
472	1112	UC	IBD	63.100	11.390	1.109	19.12
473	1114	CONTROL	NON IBD	12.280	11.390	25.052	10.00
474	1135	CONTROL	NON IBD	7.086	11.931	2.270	11.25
475	1136	CONTROL	NON IBD	11.118	11.931	1.062	67.24
476	1139	CONTROL	NON IBD	9.346	11.931	21.020	34.18
477	1141	CONTROL	NON IBD	11.973	11.931	5.227	5.49
478	1143	CONTROL	NON IBD	19.181	11.931	24.465	8.46
479	1201	CONTROL	NON IBD	8.430	11.931	11.091	40.82
480	1207	CONTROL	NON IBD	13.561	11.931	5.115	22.20

Table 1
ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person
database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
481	1208	CONTROL	NON IBD	1.500	11.931	15.423	26.45
482	1209	CONTROL	NON IBD	19.976	11.931	5.271	28.89
483	1227	UC	IBD	112.645	11.931	1.200	14.79
484	1229	CONTROL	NON IBD	12.523	11.931	36.391	23.02
485	1230	CONTROL	NON IBD	5.987	11.931	3.079	9.38
486	1303	CONTROL	NON IBD	7.880	11.931	4.410	12.96
487	1305	CONTROL	NON IBD	8.369	11.931	1.527	2.13
488	1307	CONTROL	NON IBD	9.835	11.931	9.982	17.15
489	1308	UC	IBD	20.648	11.931	19.794	10.29
490	1309	CONTROL	NON IBD	11.546	11.931	20.420	50.30
491	1323	CONTROL	NON IBD	11.607	11.931	4.345	9.15
492	1326	CONTROL	NON IBD	17.288	11.931	5.454	9.53
493	1330	CONTROL	NON IBD	5.070	11.931	4.736	20.73
494	1334	CONTROL	NON IBD	11.057	11.931	1.253	2.90
495	1408	CONTROL	NON IBD	12.034	11.931	30.089	13.11
496	1413	CONTROL	NON IBD	7.941	11.931	3.719	5.11
497	1419	CONTROL	NON IBD	11.057	11.931	3.732	82.70
498	1420	CONTROL	NON IBD	7.636	11.931	5.493	9.53
499	1427	UC	IBD	97.373	11.931	6.080	17.61
500	1436	CONTROL	NON IBD	21.503	11.931	1.840	12.50
501	1440	UC	IBD	35.858	11.931	36.443	15.85
502	1501	UC	IBD	109.312	12.074	5.806	11.36
503	1505	UC	IBD	131.869	12.074	2.518	5.95
504	1506	CD	IBD	10.623	12.074	85.530	46.27
505	1507	CD	IBD	2.230	12.074	101.096	46.27
506	1511	CD	IBD	3.738	12.074	5.741	10.29
507	1514	UC	IBD	159.279	12.074	3.510	28.28
508	1517	CD	IBD	43.869	12.074	16.310	13.95
509	1519	UC	IBD	79.475	12.074	4.684	11.28
510	1603	CD	IBD	2.623	12.074	98.904	39.94
511	1604	CD	IBD	8.197	12.074	47.651	74.09
512	1605	CD	IBD	10.557	12.074	16.127	30.03

Table 1

ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)	
5	513	1607	CD	IBD	10.623	12.074	10.626	11.28
	514	1608	CD	IBD	3.213	12.074	6.971	27.82
	515	1610	CD	IBD	6.557	12.074	0.368	0.91
	516	1611	CD	IBD	6.623	12.074	97.540	77.97
5	517	1613	CD	IBD	15.157	22.230	73.203	33.61
	518	1614	CD	IBD	22.410	22.230	16.171	16.23
	519	1616	CD	IBD	8.365	22.230	96.712	63.34
	520	1618	CD	IBD	12.164	22.230	1.454	3.28
10	521	1619	CD	IBD	7.406	22.230	54.002	27.13
	522	1620	CD	IBD	17.114	22.230	6.346	36.52
	523	1623	CONTROL	NON IBD	13.738	22.230	11.442	23.18
	524	1625	CD	IBD	21.757	22.230	0.000	8.78
	525	1627	CD	IBD	61.704	22.230	0.775	5.27
15	526	1628	CD	IBD	6.293	22.230	8.330	13.11
	527	1632	CONTROL	NON IBD	8.135	22.230	97.214	108.62
	528	1634	CD	IBD	6.370	22.230	67.536	16.35
	529	1636	CONTROL	NON IBD	7.483	22.230	0.489	6.87
20	530	1638	CONTROL	NON IBD	7.713	22.230	2.786	8.78
	531	1639	CD	IBD	12.394	22.230	0.802	9.56
	532	1640	CD	IBD	8.596	22.230	33.564	34.06
	533	1712	UC	IBD	154.144	22.230	0.000	3.39
	534	1713	CD	IBD	5.871	22.230	3.030	11
	535	1714	CD	IBD	11.704	22.230	44.775	27.16
25	536	1728	CONTROL	NON IBD	13.085	22.230	1.807	11.04
	537	1805	UC	IBD	92.748	22.230	-1.957	7.73
	538	1811	UC	IBD	25.058	22.230	5.762	21.73
	539	1812	CD	IBD	12.164	22.230	3.085	19.08
30	540	1813	CONTROL	NON IBD	5.833	22.230	-0.544	19.2
	541	1817	CONTROL	NON IBD	4.375	22.230	0.272	5.77
	542	1914	CONTROL	NON IBD	15.925	22.230	10.096	8.86
	543	1939	UC	IBD	11.282	22.230	1.508	7.96
	544	1945	CD	IBD	11.972	22.230	19.541	24.85

Table 1

ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
545	2002	UC	IBD	52.724	22.230	57.236	18.73
546	2010	UC	IBD	35.125	11.931	8.588	10.65
547	2017	CD	IBD	11.484	11.931	22.761	16.54
548	2025	UC	IBD	31.643	11.931	-0.707	7.06
549	2027	UC	IBD	92.059	11.931	7.949	23.60
550	2034	CD	IBD	11.240	11.931	76.996	32.31
551	2037	CD	IBD	102.671	20.016	2.603	11.74
552	2048	CD	IBD	19.565	20.016	34.830	117.25
553	2049	UC	IBD	125.839	20.016	3.284	18.30
554	2050	UC	IBD	63.043	20.016	3.231	4.37
555	2152	CD	IBD	32.547	20.016	10.201	
556	2203	CD	IBD	13.292	20.016	5.345	10.97
557	2205	CD	IBD	14.286	20.016	26.638	31.35
558	2228	CONTROL	NON IBD	10.870	20.016	3.668	9.05
559	2232	CD	IBD	28.882	20.016	118.812	110.53
560	2233	UC	IBD	49.814	20.016	8.245	15.17
561	2234	JC	IBD	76.211	20.016	0.000	5.56
562	2236	CONTROL	NON IBD	8.944	20.016	1.310	5.48
563	2237	CD	IBD	120.124	20.016	20.437	47.36
564	2240	CD	IBD	30.932	20.016	135.179	97.72
565	2241	CD	IBD	24.845	20.016	108.489	53.72
566	2243	CD	IBD	10.870	20.016	66.498	20.22
567	2244	CD	IBD	2.609	20.016	2.725	3.60
568	2250	UC	IBD	78.634	20.016	8.611	14.85
569	2252	UC	IBD	74.161	20.016	6.393	15.77
570	2253	UC	IBD	26.025	20.016	0.838	6.24
571	2254	CD	IBD	14.907	20.016	4.175	7.37
572	2256	CD	IBD	18.012	20.016	3.843	17.01
573	2257	CD	IBD	31.553	20.016	54.603	33.55
574	2259	CD	IBD	20.000	20.016	132.804	120.82
575	2261	CD	IBD	28.199	20.016	114.690	91.47
576	2270	NON IBD	NON IBD	20.559	20.016	11.651	7.57

Table 1
ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
5	577	2303	CD	IBD 7.329	20.016	4.000	13.81
	578	2314	UC	IBD 93.665	20.016	6.847	5.48
	579	2315	CONTROL	NON IBD 11.242	20.016	8.157	6.93
	580	2318	UC	IBD 5.031	20.016	50.515	22.18
	581	2358	CD	IBD 14.806	25.355	17.782	23.70
	582	2364	UC	IBD 32.524	25.355	4.873	8.85
	583	2368	UC	IBD 10.194	25.355	60.541	67.69
	584	2406	UC	IBD 94.053	25.355	11.913	15.33
	585	2407	CD	IBD 15.291	25.355	13.205	57.89
10	586	2408	CD	IBD 40.473	25.355	20.035	18.69
	587	2420	CD	IBD 179.612	25.355	4.419	22.38
	588	2422	CD	IBD 11.529	25.355	4.603	16.09
	589	2427	UC	IBD 13.046	25.355	3.217	10.93
	590	2429	UC	IBD 285.012	25.355	4.795	20.69
15	591	2435	UC	IBD 56.675	25.355	5.945	12.14
	592	2437	CD	IBD 12.197	25.355	2.896	22.49
	593	2438	UC	IBD 165.473	25.355	2.283	26.24
	594	2439	CD	IBD 142.901	25.355	17.880	37.63
	595	2442	UC	IBD 19.478	25.355	112.226	159.45
20	596	2447	UC	IBD 80.886	25.355	0.291	23.01
	597	2451	CD	IBD 26.699	25.355	14.003	75.11
	598	2452	UC	IBD 22.209	25.355	16.210	17.02
	599	2453	UC	IBD 123.544	25.355	51.601	51.27
	600	2454	CD	IBD 22.573	25.355	3.034	24.06
25	601	2456	UC	IBD 20.813	25.355	110.694	64.62
	602	2464	CD	IBD 62.864	25.355	64.961	146.93
	603	2466	CD	IBD 21.905	25.355	31.592	88.16
	604	2467	UC	IBD 41.080	25.355	3.080	23.54
	605	2473	CD	IBD 21.516	12.657	21.327	15.52
30	606	2475	UC	IBD 21.890	12.657	17.497	54.27
	607	2477	UC	IBD 46.716	12.657	3.049	23.09
	608	2503	UC	IBD 30.112	12.657	1.593	10.72

Table 1
ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
609	2506	UC	IBD	71.276	12.657	0.919	16.19
610	2507	UC	IBD	76.241	12.657	7.783	35.01
611	2509	CD	IBD	13.454	12.657	84.036	80.88
612	2510	CD	IBD	16.231	12.657	107.722	138.91
613	2511	CD	IBD	16.551	12.657	25.770	23.46
614	2514	UC	IBD	24.933	12.657	0.000	12.59
615	2516	UC	IBD	104.965	12.657	0.797	10.49
616	2517	UC	IBD	38.174	12.657	2.237	32.01
617	2520	CD	IBD	23.599	12.657	9.622	21.06
618	2521	UC	IBD	40.790	12.657	88.096	88.98
619	2522	UC	IBD	24.372	13.499	1.731	21.96
620	2533	UC	IBD	39.349	12.657	1.593	15.22
621	2535	CD	IBD	9.343	12.657	0.950	6.45
622	2537	UC	IBD	69.301	12.657	0.000	4.57
623	2538	CD	IBD	4.849	20.382	2.978	7.5
624	2540	CD	IBD	10.358	12.657	102.712	132.08
625	2541	UC	IBD	8.596	12.657	2.405	16.49
626	2542	UC	IBD	38.334	12.657	5.592	19.85
627	2543	UC	IBD	150.721	20.382	7.531	24.76
628	2555	CD	IBD	96.003	20.382	0.915	14.87
629	2557	UC	IBD	16.710	20.382	1.774	11.76
630	2559	UC	IBD	140.105	20.382	5.912	20.75
631	2560	CD	IBD	9.174	20.382	7.573	11.7
632	2561	CD	IBD	5.177	20.382	3.533	23.51
633	2562	CD	IBD	6.750	20.382	10.206	8.71
634	2563	CD	IBD	17.369	20.382	16.258	6.78
635	2564	UC	IBD	8.257	20.382	0.479	5.05
636	2569	CD	IBD	29.030	20.382	5.870	24.2
637	2570	UC	IBD	9.183	12.657	2.365	11.89
638	2572	UC	IBD	109.877	12.657	83.784	39.83
639	2573	UC	IBD	71.223	12.657	3.773	39.56
640	2575	CD	IBD	15.750	12.657	6.461	12.45

Table 1
ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
5	641	2576	UC	IBD 65.350	12.657	1.478	16.39
	642	2577	CD	IBD 11.426	12.657	101.788	22.75
	643	2578	CD	IBD 21.244	13.499	82.630	51.45
	644	2579	UC	IBD 68.830	13.499	0.338	4.36
10	645	2601	UC	IBD 76.555	13.499	9.333	14.25
	646	2602	CD	IBD 5.253	13.499	45.861	31.54
	647	2605	UC	IBD 129.703	13.499	1.408	7.75
	648	2607	UC	IBD 66.242	13.499	3.125	7.05
	649	2608	UC	IBD 16.261	13.499	4.842	7.12
15	650	2609	CD	IBD 13.055	13.499	26.675	34.92
	651	2610	CD	IBD 7.532	13.499	2.717	10.58
	652	2611	UC	IBD 13.210	13.499	1.182	8.78
	653	2612	CD	IBD 146.505	13.499	3.041	20.06
20	654	880585	CONTROL	NON IBD 12.536	8.283	1.244	28.8184
	655	890037	CONTROL	NON IBD 5.364	8.283	1.168	13.5446
	656	890361	CONTROL	NON IBD 6.356	8.283	1.206	3.8904
	657	890550	CONTROL	NON IBD 3.907	8.283	8.517	8.9337
25	658	890581	CONTROL	NON IBD 14.985	8.283	2.450	4.755
	659	890622	CONTROL	NON IBD 9.271	8.283	2.902	4.1786
	660	890627	CONTROL	NON IBD 17.318	8.283	31.242	22.9106
	661	900097	CONTROL	NON IBD 7.910	9.503	2.713	8.5014
	662	900146	CONTROL	NON IBD 5.726	9.503	0.735	7.7809
	663	900224	CONTROL	NON IBD 19.540	9.503	1.865	10.6628
30	664	900329	CONTROL	NON IBD 9.504	9.503	1.489	2.7377
	665	900421	CONTROL	NON IBD 7.202	9.503	14.754	12.1037
	666	900450	CONTROL	NON IBD 6.907	9.503	0.471	4.3227
	667	900452	CONTROL	NON IBD 6.789	9.503	1.131	17.8674
	668	900482	CONTROL	NON IBD 11.452	9.503	6.425	19.1642
	669	900504	CONTROL	NON IBD 12.220	9.503	0.343	2.8184
	670	900659	CONTROL	NON IBD 14.699	9.503	4.250	11.9503
	671	900709	CONTROL	NON IBD 16.588	9.503	1.202	10.0338
	672	900748	CONTROL	NON IBD 7.792	9.503	1.524	4.735

Table 1
ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person
database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
5	673	910039	CONTROL	NON IBD 4.723	9.503	3.713	5.2987
	674	910042	CONTROL	NON IBD 4.959	9.503	3.155	30.5524
	675	910056	CONTROL	NON IBD 13.872	9.503	1.760	13.3032
	676	910095	CONTROL	NON IBD 14.876	9.503	0.515	11.7249
10	677	910101	CONTROL	NON IBD 13.991	9.503	2.576	30.5524
	678	910104	CONTROL	NON IBD 17.119	9.503	3.069	3.6076
	679	910108	CONTROL	NON IBD 9.622	9.503	1.717	6.3134
	680	910156	CONTROL	NON IBD 3.011	9.503	1.996	6.5388
	681	910214	CONTROL	NON IBD 6.966	9.503	0.622	7.779
15	682	910217	CONTROL	NON IBD 4.604	9.503	0.880	5.9751
	683	910220	CONTROL	NON IBD 12.633	9.503	3.069	15.558
	684	910234	CONTROL	NON IBD 12.279	9.503	5.216	117.1364
	685	910561	CONTROL	NON IBD 11.393	9.503	5.646	2.2099
20	686	920028	CONTROL	NON IBD 15.821	9.503	1.695	11.5172
	687	920056	CONTROL	NON IBD 12.102	9.503	2.284	4.2073
	688	920142	CONTROL	NON IBD 9.858	9.503	5.789	36.2091
	689	920184	CONTROL	NON IBD 9.792	11.138	7.163	6.3545
25	690	920258	CONTROL	NON IBD 4.036	11.138	4.077	9.0969
	691	920260	CONTROL	NON IBD 6.706	11.138	4.481	6.2207
	692	920264	CONTROL	NON IBD 9.080	11.138	3.343	3.5451
	693	920302	CONTROL	NON IBD 6.944	11.138	4.555	6.1538
	694	920346	CONTROL	NON IBD 12.226	11.138	10.670	52.3745
30	695	920448	CONTROL	NON IBD 10.564	11.138	4.353	5.3511
	696	930031	CONTROL	NON IBD 12.997	11.138	1.635	5.9531
	697	930182	CONTROL	NON IBD 4.392	11.138	5.197	12.9097
	698	930184	CONTROL	NON IBD 2.967	11.138	2.204	31.505
	699	930219	CONTROL	NON IBD 11.157	11.138	1.157	12.1739
	700	930222	CONTROL	NON IBD 11.039	11.138	14.123	18.2608
	701	930225	CONTROL	NON IBD 4.392	11.138	2.755	10.903
	702	930228	CONTROL	NON IBD 2.849	11.138	4.463	7.4916
	703	930243	CONTROL	NON IBD 10.148	11.138	0.680	9.3645
	704	930266	CONTROL	NON IBD 4.273	11.138	9.605	16.321

Table 1
ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
5	705	930268	CONTROL	NON IBD 11.988	11.138	14.509	14.9163
	706	930516	CONTROL	NON IBD 3.680	11.138	14.527	5.5518
	707	940106	CONTROL	NON IBD 7.596	11.138	1.451	3.4782
	708	950795	CONTROL	NON IBD 3.442	11.138	10.854	9.6321
	709	950940	CONTROL	NON IBD 1.068	11.138	1.157	4.0133
10	710	860211	CONTROL	NON IBD 9.489	11.205	4.615	10.0094
	711	860214	CONTROL	NON IBD 7.688	11.205	0.671	3.0217
	712	870018	CONTROL	NON IBD 7.147	11.205	0.378	22.0018
	713	880055	CONTROL	NON IBD 6.907	11.205	0.378	3.4938
	714	880071	CONTROL	NON IBD 4.384	11.205	5.538	7.7431
	715	880626	CONTROL	NON IBD 11.832	11.205	3.923	25.118
	716	890061	CONTROL	NON IBD 4.745	11.205	0.441	1.8885
	717	890163	CONTROL	NON IBD 8.468	11.205	5.496	31.1614
	718	890308	CONTROL	NON IBD 8.724	8.754	0.399	2.2662
15	719	890353	CONTROL	NON IBD 15.608	8.754	3.147	6.3267
	720	890362	CONTROL	NON IBD 7.003	8.754	1.575	14.1772
	721	890516	CONTROL	NON IBD 12.819	8.754	1.877	29.9578
	722	890519	CONTROL	NON IBD 9.318	8.754	1.381	3.4599
	723	890523	CONTROL	NON IBD 3.323	8.754	4.078	13.2489
20	724	890529	CONTROL	NON IBD 21.899	8.754	1.251	2.8691
	725	900560	CONTROL	NON IBD 6.915	8.558	4.746	22.1097
	726	900606	CONTROL	NON IBD 5.230	8.558	0.604	26.4978
	727	900608	CONTROL	NON IBD 14.991	8.558	0.820	6.5822
	728	910164	CONTROL	NON IBD 19.349	8.558	36.980	13.924
25	729	920551	CONTROL	NON IBD 8.731	10.984	9.250	8.0612
	730	920552	CONTROL	NON IBD 11.369	10.984	8.989	42.5
	731	920584	CONTROL	NON IBD 5.088	10.984	1.024	14.4786
	732	920748	CONTROL	NON IBD 9.381	11.947	29.980	49.2873
	733	921032	CONTROL	NON IBD 10.727	11.947	0.422	4.051
30	734	930026	CONTROL	NON IBD 6.867	11.947	4.518	2.7006
	735	930055	CONTROL	NON IBD 6.499	11.947	6.928	23.0307
	736	930077	CCNTROL	NON IBD 10.791	11.947	0.086	26.2565

Table 1
 ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
5	737 930130	CONTROL	NON IBD	7.848	11.947	0.964	4.201
	738 930138	CONTROL	NON IBD	14.654	11.947	1.406	7.5018
	739 930230	CONTROL	NON IBD	5.886	11.947	3.112	7.4268
	740 930252	CONTROL	NON IBD	16.048	13.486	1.667	12.378
	741 930288	CONTROL	NON IBD	11.916	13.486	0.321	5.0262
10	742 930300	CONTROL	NON IBD	12.994	13.486	1.847	10.3525
	743 930446	CONTROL	NON IBD	13.713	13.486	3.412	13.6909
	744 930559	CONTROL	NON IBD	9.566	11.473	0.796	29.5436
	745 930666	CONTROL	NON IBD	5.489	11.473	8.599	17.324
	746 930804	CONTROL	NON IBD	8.987	10.373	0.994	2.2428
	747 930838	CONTROL	NON IBD	9.590	10.373	24.645	18.1497
	748 930875	CONTROL	NON IBD	6.553	12.139	1.754	6.9209
	749 930877	CONTROL	NON IBD	6.382	12.139	0.456	22.1751
15	750 930924	CONTROL	NON IBD	8.091	12.139	4.438	7.6977
	751 930925	CONTROL	NON IBD	9.174	12.139	1.123	9.5338
	752 930977	CONTROL	NON IBD	7.806	12.139	1.772	11.0169
	753 86-0034 S	CONTROL	NON IBD	8.367	10.951	6.390	16.0142
	754 86-0074 S	CONTROL	NON IBD	14.490	10.951	3.465	18.6239
20	755 86-0085 S	CONTROL	NON IBD	5.306	9.812	2.776	10.1486
	756 86-0126 S	CONTROL	NON IBD	14.552	9.115	1.036	11.0536
	757 87-0005 S	CONTROL	NON IBD	11.293	10.951	14.048	12.9369
	758 87-0022 S	CONTROL	NON IBD	2.734	10.815	2.955	-2.397
	759 87-0068 S	CONTROL	NON IBD	6.064	10.815	4.183	4.9438

Table 1
ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
5	760 87-0092 S	CONTROL	NON IBD	10.544	10.951	5.361	23.9855
761 87-0159 S	CONTROL	NON IBD	14.959	11.895	1.408	9.736	
762 87-0292 S	CONTROL	NON IBD	8.186	9.812	2.916	7.8651	
763 87-0294 S	CONTROL	NON IBD	6.620	9.812	1.611	10.1612	
764 88-0250 S	CONTROL	NON IBD	4.274	11.895	1.001	4.1642	
765 88-0280 S	CONTROL	NON IBD	9.456	10.951	5.224	75.0296	
766 88-0397 S	CONTROL	NON IBD	6.361	12.672	2.793	5.5443	
767 88-0448 S	CONTROL	NON IBD	8.097	10.815	3.349	-4.1947	
768 88-0555 S	CONTROL	NON IBD	10.971	10.815	15.416	1.4981	
10	769 88-0658 S	CONTROL	NON IBD	10.340	10.951	4.371	9.4899
770 88-0662 S	CONTROL	NON IBD	18.035	9.115	1.376	4.9228	
771 89-0683 S	CONTROL	NON IBD	12.932	11.895	4.715	10.1466	
772 90-0136 S	CONTROL	NON IBD	12.585	10.951	10.076	23.0614	
773 90-0180 S	CONTROL	NON IBD	9.674	10.815	1.784	146.1423	

Table 1

ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
5	774 90-0218 S	CONTROL	NON IBD	10.166	12.672	2.099	11.2495
	775 90-0233 S	CONTROL	NON IBD	15.872	15.179	7.081	18.9512
	776 90-0255 S	CONTROL	NON IBD	17.951	15.179	3.666	13.5234
	777 90-0261 S	CONTROL	NON IBD	22.229	9.467	18.784	33.6706
	778 90-0286 S	CONTROL	NON IBD	7.692	12.816	0.377	1.319
	779 90-0302 S	CONTROL	NON IBD	8.590	9.812	5.422	4.137
	780 90-0315 S	CONTROL	NON IBD	19.154	9.115	5.736	7.0458
	781 90-0335 S	CONTROL	NON IBD	9.826	9.115	2.699	5.338
	782 90-0703 S	CONTROL	NON IBD	7.669	12.672	30.082	25.7934
	783 90-0734 S	CONTROL	NON IBD	19.593	10.815	2.018	6.0674
10	784 91-0267 S	CONTROL	NON IBD	28.027	10.951	3.604	18.8612
	785 91-0484 S	CONTROL	NON IBD	6.660	10.815	9.683	11.2359
	786 92-1001 S	CONTROL	NON IBD	6.013	9.812	5.477	2.3917
	787 92-329 S	CONTROL	NON IBD	10.821	12.816	4.353	5.7156

Table 1
ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)	
788	92-404 S	CONTROL	NON IBD	90.045	9.812	3.555	3.6638	
789	92-407 S	CONTROL	NON IBD	12.279	9.812	1.016	3.5661	
790	92-466 S	CONTROL	NON IBD	5.104	9.812	6.863	8.549	
791	92-702 S	CONTROL	NON IBD	5.328	10.815	5.953	8.6891	
5	792	92-809 S	CONTROL	NON IBD	12.164	11.895	0.831	12.6099
793	92-832 S	CONTROL	NON IBD	11.973	10.951	6.217	8.5978	
794	92-900 S	CONTROL	NON IBD	10.450	10.803	2.375	20.0000	
795	92-9721 S	CONTROL	NON IBD	5.272	10.278	3.140	11.7276	
10	796	92-988 S	CONTROL	NON IBD	14.490	9.115	15.896	33.2256
797	93-0487 S	CONTROL	NON IBD	16.849	17.859	9.829	4.6658	
798	93-0509 S	CONTROL	NON IBD	3.077	12.816	0.363	2.0517	
799	93-0654 S	CONTROL	NON IBD	27.709	12.980	13.160	13.4641	
800	93-0741 S	CONTROL	NON IBD	10.338	17.859	1.483	12.6984	
801	93-0746 S	CONTROL	NON IBD	8.388	9.812	4.745	8.6468	

Table 1
ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
5	802 93- 0768 S	CONTROL	NON IBD	10.410	12.816	1.165	3.4359
	803 93- 0996 S	CONTROL	NON IBD	7.291	10.815	2.077	-2.0224
	804 93- 1001 S	CONTROL	NON IBD	19.728	10.951	2.873	22.8351
	805 93- 1010 S	CONTROL	NON IBD	4.629	9.467	4.406	8.3891
	806 93- 1039 S	CONTROL	NON IBD	5.029	9.467	1.478	2.4096
	807 94- 0017 S	CONTROL	NON IBD	26.288	10.815	3.042	17.2284
	808 94- 0083 S	CONTROL	NON IBD	6.015	10.803	7.010	7.5294
	809 94- 0095 S	CONTROL	NON IBD	9.990	15.179	1.394	4.5078
	810 94- 0104 S	CONTROL	NON IBD	4.343	9.467	39.441	25.3458
	811 94- 0143 S	CONTROL	NON IBD	23.365	12.672	15.723	20.8211
	812 94- 0181 S	CONTROL	NON IBD	11.567	9.115	1.585	8.185
	813 94- 0189 S	CONTROL	NON IBD	7.967	12.672	0.220	3.695
	814 94- 0228 S	CONTROL	NON IBD	13.767	10.278	2.342	10.0392
	815 94- 0237 S	CONTROL	NON IBD	13.205	11.895	7.395	20.9384

Table 1
ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
5	816 94- 0245 S	CONTROL	NON IBD	9.744	12.816	2.684	11.2848
	817 94- 0301 S	CONTROL	NON IBD	15.676	12.980	3.221	14.8173
	818 94- 0308 S	CONTROL	NON IBD	9.200	9.467	2.001	11.3788
	819 94- 0384 S	CONTROL	NON IBD	14.513	17.859	1.507	12.0428
	820 94- 0459 S	CONTROL	NON IBD	30.599	10.815	8.688	12.1348
	821 94- 0466 S	CONTROL	NON IBD	12.752	12.980	1.527	1.6914
	822 94- 0467 S	CONTROL	NON IBD	4.686	9.467	19.113	27.9668
	823 94- 0550 S	CONTROL	NON IBD	5.314	9.467	2.554	35.4306
	824 94- 0569 S	CONTROL	NON IBD	7.551	12.672	5.684	13.2985
	825 94- 0635 S	CONTROL	NON IBD	9.333	12.816	7.079	25.5243
	826 94- 0655 S	CONTROL	NON IBD	8.939	10.238	1.987	4.4011
	827 94- 0719 S	CONTROL	NON IBD	16.347	12.980	17.939	0.6089
	828 94- 0727 S	CONTROL	NON IBD	6.171	9.467	5.929	9.5046
	829 94- 0794 S	CONTROL	NON IBD	16.836	15.179	3.807	18.8592

Table 1
 ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)
	830 95-0029 S	CONTROL	NON IBD	4.101	10.278	9.122	38.5245
5	831 95-0033 S	CONTROL	NON IBD	9.959	10.278	2.324	16.5195
	832 95-0073 S	CONTROL	NON IBD	9.888	15.179	4.261	3.4958
	833 95-0108 S	CONTROL	NON IBD	14.400	9.467	2.330	3.1236
	834 95-0109 S	CONTROL	NON IBD	20.086	12.980	16.321	20.0947
	835 95-0114 S	CONTROL	NON IBD	14.503	15.179	4.935	9.4756
10	836 95-0181 S	CONTROL	NON IBD	4.114	9.467	2.479	7.0058
	837 95-0191 S	CONTROL	NON IBD	12.697	10.803	3.206	6.9019
	838 95-0192 S	CONTROL	NON IBD	13.943	9.467	6.173	14.4434
	839 95-0225 S	CONTROL	NON IBD	16.826	12.980	4.229	2.7063
	840 95-0275 S	CONTROL	NON IBD	4.469	10.238	4.968	7.1428
	841 95-0338 S	CONTROL	NON IBD	5.858	10.278	10.159	8.9533
	842 95-0554 S	CONTROL	NON IBD	2.855	10.803	1.977	17.3333
	843 95-0558 S	CONTROL	NON IBD	11.891	10.238	1.758	17.8932

Table 1

ANCA, ASCA-IgA and ASCA-IgG serology data from 851 person database

COUNT	SAMPLE ID	DIAGNOSIS	IBD CLASS	ANCA ELISA RESULT	ANCA ELISA CUT-OFF	ASCA-IgA RESULT (EU)	ASCA-IgG RESULT (EU)	
844	95-0684 S	CONTROL	NON IBD	29.657	9.467	0.862	26.1269	
845	95-0716 S	CONTROL	NON IBD	8.006	12.980	8.977	9.4046	
846	95-0880 S	CONTROL	NON IBD	17.029	9.467	14.742	12.0515	
847	95-0887 S	CONTROL	NON IBD	7.095	12.980	10.992	30.9201	
5	848	95-1012 S	CONTROL	NON IBD	13.998	12.980	9.420	30.5818
	849	95-1038 S	CONTROL	NON IBD	15.159	17.859	7.097	26.2295
	850	95-1077 S	CONTROL	NON IBD	14.066	17.859	2.858	4.7919
	851	96-0107 S	CONTROL	NON IBD	9.197	10.278	3.339	7.9215

The individuals described in Table 1 were classed in one of several disease or control categories. As shown in Table 2, of the 851 total patients, 433 (50.88%) were in the IBD category and 418 (49.12%) were in the Non-IBD category. All serum samples were tested by neutrophil ELISA and for immunoglobulin G and immunoglobulin A antibodies to mannan from *Saccharomyces cerevisiae uvarum* as described in Example I. Neutrophil ELISA positive samples were additionally analyzed by immunofluorescence assay with neutrophil substrate, followed by DNase

treatment for immunofluorescence positive samples that show a perinuclear pattern. No other measurements were made on the samples.

Table 2 Inflammatory Bowel Disease Database		
Disease or Control Category	Number of Patients	Percent of Total
IBD Category		
Crohn's disease*	218	25.62
Ulcerative colitis*	212	24.91
Ulcerative colitis/PSC*	3	0.35%
IBD Subtotal	433	50.88
Non-IBD category		
Disease control*	60	7.05
Non-IBD*	35	4.11
IBS*	22	2.59
Wegener's granulomatosis*	1	0.12
Control	300	35.35
Non-IBD subtotal	418	49.12
Total	851	100
* verified by colonoscopy, radiology and/or histology		

B. Simultaneous variation of ANCA, ASCA-IgA and ASCA-IgG cut-off values

The three different ELISA cut-off values for ANCA reactivity, ASCA-IgA reactivity and ASCA-IgG reactivity were varied simultaneously. Base cut-off values were determined as follows:

5 To determine the base cut-off value for ANCA reactivity, a panel of twenty verified negative control samples was used with a calibrator with a defined ELISA Unit (EU) value. The base positive/negative cut-off for each ELISA run was defined as the optical density (OD) of 10 the Calibrator minus the mean (OD) value for the panel of twenty negatives (plus 2 standard deviations) times the EU value of the Calibrator. The base cut-off value for ANCA reactivity was therefore about 10 to 20 EU, with any patient sample having an average EU value greater than 15 the base cut-off marked as ELISA positive for ANCA reactivity. Similarly, a patient sample having an average EU value is less than or equal to the base cut-off was determined to be negative for ANCA reactivity.

20 To determine the base cut-off value for ASCA-IgA and ASCA-IgG, single point calibrators having fixed EU values were used. OD values for patient samples were compared to the OD value for the Calibrators and multiplied by the Calibrator assigned values. The base 25 cut-off value for the ASCA-IgA ELISA was 20 EU. The base cut-off value for the ASCA-IgG ELISA was 40 EU.

Using this existing set of test data for 851 patients having IBD status determined by colonoscopy or radiology or both or who were asymptomatic controls, the 30 three cut-off values were simultaneously adjusted to observe the effects on clinical parameters: sensitivity, specificity, negative predictive value, positive

predictive value, and overall agreement. In particular, design of Experiments (DOE) methodology was used to simultaneously vary the three cut-off ELISA values and to determine the effects on the resulting clinical 5 parameters of sensitivity, specificity, negative predictive value, positive predictive value and overall agreement. The DOE methodology is advantageous in that variables are tested in a nested array requiring fewer runs and identifying cooperative interactions among the 10 three cut-off variables. Optimization software DOE Keep It Simple Statistically (KISS) was obtained from Air Academy Associates (Colorado Springs, CO) and used to assign experimental runs and perform the simultaneous equation calculations.

15 Cut-off values were varied as set forth in Table 3 below:

Table 3			
ELISA	Low Cut-off	Standard	High Cut-off
	Value	Cut-off Value	Value
ANCA	0.5X Standard	1.0X Standard	1.5X Standard
ASCA-IgA	10 EU	20 EU	30 EU
ASCA-IgG	20 EU	40 EU	60 EU

20 A three variable (ANCA cut-off, ASCA-IgA cut-off, ASCA-IgG cut-off) and three level (low, middle, and high; see Table 3) central composite (CCD) factorial 25 design experiment was conducted as follows. In each experiment listed, the cut-off values for each of the three ELISA tests were set as shown in the first column of Table 4. Analysis using the KISS program was made with all first, second and third order variables

operable. The first experiment shown in row 1 of Table 4, for example, indicates a cut-off value of ANCA = 0.5, ASCA-IgA = 10, and ASCA-IgG = 20. By comparison with these assigned cut-off values, the test results 5 determined for all of the 851 samples in the data base were assigned as true positive, true negative, false positive, or false negative. Using these results and the clinically defined diagnosis, sensitivity, specificity, overall agreement, positive predictive value, and 10 negative predictive value were calculated. Using the DOE KISS program, optimized sets of cut-off values for selected clinical parameters were calculated.

The clinical parameter results for each set of three cut-off variables are shown in Table 4. Although 15 these results are the calculated points determined by the experimental design, clinical parameter results for any other set of cut-offs within the cut-off boundaries also can be calculated. The three dimensional test box determined by the extremes of the three variables defines 20 the region in which testing was conducted. These results show that there is a continuum of solutions of clinical responses within the boundaries of the cut-off values and that the DOE methodology can be used to determine the sets of cut-off values which present the most useful 25 clinical parameters for a particular patient population.

Table 4
 Clinical Parameter Results from Simultaneous Variation
 of ANCA, ASCA-IgA, and ASCA-IgG cut-off values X, Y,
 and Z in a population with 50% disease prevalence

	Cut-offs*	% Sens.	% Spec.	% Overall Agreement	% PPV	% NPV
		96.3	13.6	55.7	60.5	78.1
5	0.5/10/60	95.2	14.8	55.7	53.6	74.7
	0.5/30/20	96.1	14.4	55.9	53.8	77.9
	0.5/30/60	94.9	16.3	53.3	54.0	75.6
	1.5/10/20	81.3	64.6	73.1	70.4	76.9
	1.5/10/60	77.1	75.4	76.3	76.4	76.1
	1.5/30/20	78.5	70.8	74.4	73.6	76.1
	1.5/30/60	69.5	86.1	77.7	83.7	73.2
	1.0/20/40	82.2	67.3	74.9	72.2	78.5
	1.0/20/40	82.2	67.3	74.9	72.2	78.5
	0.5/20/40	94.9	16.3	56.3	54.0	75.6
10	1.5/20/40	74.1	83.5	78.7	82.3	75.7
	1.0/10/40	85.0	61.0	73.2	69.3	79.7
	1.0/30/40	80.6	57.4	69.2	66.2	74.1
	1.0/20/20	85.2	57.7	71.7	67.6	79.0
	1.0/20/60	81.8	67.5	74.7	72.2	78.1

* ANCA/ASCA-IgA/ASCA-IgG cut-offs

The maximum possible sensitivity, specificity,
 25 negative predictive value, positive predictive value and
 overall agreement within the range of ANCA values (0.5X
 to 1.5X standard); ASCA-IgA values (10 To 30 ELISA units)
 and ASCA-IgG values (20 to 60 ELISA units) were
 determined with the entire 851 person database having an
 30 IBD disease prevalence of 50%. The results are shown in
 Table 5.

Table 5
Maximum possible clinical parameters in a population
having an IBD disease prevalence of 50%

5 Clinical Parameters (N=851)	Maximum possible correlation	Cut-off values
		ANCA/ASCA-IgA/ASCA-IgG
Sensitivity	96.61%	0.50; 10.00; 20
Specificity	87.57%	1.50; 24.48; 60
Negative predictive value	80.25	0.90; 14.21; 20
Positive predictive value	84.54	1.50; 26.10; 60
Overall agreement	79.57%	1.46; 20.42; 60

The results shown in Table 5 give the maximum
15 possible clinical parameters within the ranges of cut-off
values explored in a population with an IBD disease
prevalence of 50%. For example, the highest possible
sensitivity is 96.61% and is obtained with an ANCA
20 cut-off of 0.5, an ASCA-IgA cut-off of 10 EU, and an
ASCA-IgG cut-off of 20 EU. At this high sensitivity,
specificity is reduced, being only 13.16% at this
cut-off. These results demonstrate that ANCA, ASCA-IgA
and ASCA-IgG values can be determined to give maximum
sensitivity, but that other cut-off values are needed to
25 yield maximum specificity.

C. Determination of ANCA, ASCA-IgA and ASCA-IgG cut-off values for high sensitivity

Sensitivity is the fraction of all those with IBD who are diagnosed positive for IBD with the first 5 step assay. Values were selected that produced a high sensitivity (90.3%) while still maintaining a relatively high specificity. In particular, 90.3% sensitivity was achieved by setting the ANCA cut-off at 0.7 multiplied by two standard deviations above the background value of 10 ANCA-negative UC sera, ASCA-IgA cut-off at 12 EU and the ASCA-IgG cut-off at 60 EU (see Table 6). These cut-offs are distinct from the cut-offs used in the UC*Dx-1 and CD*Dx-1 assays, which are 1.0, 20, and 60, respectively.

Table 6

15 Evaluation of results with high sensitivity assay having ANCA cut-off = 0.7, ASCA-IgA cut-off = 12 EU and an ASCA-IgG cut-off = 60 EU.

	True IBD Positive	True IBD Negative	Totals
First step assay positive	391	262	653
First Step assay negative	42	156	198
Totals	433	418	851

25 With an ANCA cut-off of 0.7 multiplied by two standard deviations above the background value of ANCA-negative UC sera, an ASCA-IgA cut-off of 12 EU, and an ASCA-IgG cut-off of 60 EU, the specificity was determined to be 37.3%. Using these cut-off values and 30 the entire 851 patient database (having an IBD disease prevalence of 50%), the negative predictive value was 78.8%, the positive predictive value was 59.9%, and the

overall agreement was 64.3%. These data also can be modeled for an IBD prevalence of 15%, which represents the approximate IBD disease prevalence in a gastroenterologist's office population (see Table 7). In 5 a population having an IBD prevalence of 15%, an ANCA cut-off of 0.7 multiplied by two standard deviations above the background value of ANCA-negative UC sera, an ASCA-IgA cut-off of 12 EU and an ASCA-IgG cut-off of 60 EU resulted in a negative predictive value of 95.6%, a 10 positive predictive value of 20.3%, and overall agreement of 45.3%. The calculated performance at 15% IBD prevalence was confirmed by randomly choosing patients from the n = 851 database to construct and analyze a new database (n = 277) that had a 15% IBD prevalence.

15

Table 7

Clinical parameters with 50%, 15% and 1% IBD disease prevalence for assays run with an ANCA cut-off = 0.7, an ASCA-IgA cut-off = 12 EU and an ASCA-IgG cut-off = 60 EU

20

Clinical parameter	Disease prevalence		
	50%	15%	1%
Sensitivity	90.3%	90.3%	90.3%
Specificity	37.3%	37.3%	37.3%
Negative predictive value	78.8%	95.6%	99.7%
Positive predictive value	59.9%	20.3%	1.43%
Overall agreement	64.3%	45.3%	37.8%

25

EXAMPLE III

USE OF THE SENSITIVE 'FIRST STEP' METHOD IN COMBINATION WITH SUBSEQUENT, SPECIFIC DIAGNOSTIC ASSAYS

This example demonstrates that the "First Step" diagnostic method can be used in combination with subsequent, specific diagnostic assays such as the UC*Dx-1 and CD*Dx-1 assays.

Samples which were positive by "First Step" analysis (including true positive and false positive samples) were subsequently tested by the UC*Dx-1 and CD*Dx-1 assays, which are specific for ulcerative colitis and Crohn's disease, respectively. The results are shown in Table 8.

Table 8

15 Results of reflex of samples positive by "First Step" diagnosis to the UC*Dx-1 and CD*Dx-1 assays

diagnosis to the UC*Dx-1 and CD*Dx-1 assays			
	True IBD Positive	True IBD Negative	Totals
CD*Dx-1 and/or UC*Dx-1 Positive	287	38	325
CD*Dx-1 and/or UC*Dx-1 Negative	146	380	526
Totals	433	418	851

Together, reflex of samples positive by "First Step" analysis to the UC*Dx-1 and CD*Dx-1 performs with 25 66.3% sensitivity, 90.9% specificity, 72.2% negative predictive value, 88.3% positive predictive value and 78.4% overall agreement. These results indicate that subsequent analysis of positive samples can amplify on the initial "First Step" result. These results indicate 30 that the "First Step" diagnostic can be used, if desired,

in combination with a subsequent, more specific diagnostic method.

All journal article, reference and patent citations provided above, in parentheses or otherwise, 5 whether previously stated or not, are incorporated herein by reference in their entirety.

Although the invention has been described with reference to the examples provided above, it should be understood that various modifications can be made without 10 departing from the spirit of the invention. Accordingly, the invention is limited only by the claims.

We claim:

1. A highly sensitive method of diagnosing inflammatory bowel disease (IBD) in an individual, comprising the steps of:
 - (a) isolating a sample from said individual;
 - (b) determining by non-histological means whether said sample is positive for anti-neutrophil cytoplasmic antibodies (ANCA);
 - (c) determining whether said sample is positive for anti-*Saccharomyces cerevisiae* immunoglobulin A (ASCA-IgA);
 - (d) determining whether said sample is positive for anti-*Saccharomyces cerevisiae* immunoglobulin G (ASCA-IgG); and
 - (e) diagnosing said individual as having IBD when said sample is positive for ANCA, ASCA-IgA or ASCA-IgG, and diagnosing said individual as not having IBD when said sample is negative for ANCA, ASCA-IgA and ASCA-IgG,
provided that said method does not include histological analysis of neutrophils.
2. The method of claim 1, said method consisting of steps (a), (b), (c), (d) and (e).

- 25 3. The method of claim 1, wherein ANCA, ASCA-IgA and ASCA-IgG positivity are determined using an immunoassay.

4. A highly sensitive method of diagnosing IBD in an individual, comprising the steps of:

- (a) isolating a sample from said individual;
- (b) determining by non-histological means 5 whether said sample has an ANCA level above an ANCA cut-off value (X);
- (c) determining whether said sample has an ASCA-IgA level above an ASCA-IgA cut-off value (Y);
- (d) determining whether said sample has an 10 ASCA-IgG level above an ASCA-IgG cut-off value (Z); and
- (e) diagnosing said individual as having IBD when

said ANCA level is above X,
said ASCA-IgA level is above Y, or
15 said ASCA-IgG level is above Z,
and diagnosing said individual as not having IBD when
said ANCA level is below X,
said ASCA-IgA level is below Y,
and said ASCA-IgG value is below Z,
20 wherein X, Y, and Z are independently selected
to achieve an optimized clinical parameter selected from
the group consisting of: sensitivity, specificity,
negative predictive value, positive predictive value and
overall agreement,
25 provided that said method does not include
histological analysis of neutrophils.

5. The method of claim 4, wherein X, Y and Z are independently selected such that the sensitivity of diagnosing an individual with IBD is at least about 70%.

30 6. The method of claim 5, wherein X, Y and Z are independently selected such that the specificity of diagnosing an individual with IBD is 30-60%.

7. The method of claim 6, wherein X, Y and Z are independently selected such that the negative predictive value in a population having an IBD disease prevalence of about 15% is at least about 90%.

5 8. The method of claim 7, wherein said negative predictive value is at least about 95%.

9. The method of claim 4, wherein X, Y and Z are independently selected such that the sensitivity of diagnosing an individual with IBD is at least about 90%.

10 10. The method of claim 9, wherein X, Y and Z are independently selected such that the specificity of diagnosing an individual with IBD is 20-60%.

11. The method of claim 10, wherein X, Y and Z are independently selected such that the negative predictive value in a population having an IBD disease prevalence of about 15% is at least about 90%.

12. The method of claim 11, wherein said negative predictive value is at least about 95%.

13. The method of claim 12, wherein X, Y and Z are independently selected such that the sensitivity of diagnosing an individual with IBD is about 90%, the specificity is about 37% and the negative predictive 5 value in a population having an IBD disease prevalence of about 15% is about 95%.

14. The method of claim 4, wherein X is selected to be 0.7 multiplied by two standard deviations above the background value of 10 ANCA-negative UC sera; Y is selected to be 12 ELISA units; and Z is selected to be 60 ELISA units.

15. The method of claim 4, wherein X, Y and Z are independently selected such that the negative 15 predictive value in a patient population having an IBD disease prevalence of about 15% is at least about 95%.

16. The method of claim 4, said method consisting of steps (a), (b), (c), (d) and (e).

17. The method of claim 4, wherein said ANCA 20 level, ASCA-IgA level and ASCA-IgG level each is determined using an immunoassay.

18. The method of claim 4, comprising the steps of:

- (a) isolating a sample from said individual;
- (b) contacting an appropriate dilution of said sample with antigen specific for ANCA under conditions suitable to form a first complex of antigen specific for ANCA and ANCA;
- (c) contacting an appropriate dilution of said sample with antigen specific for ASCA under conditions suitable to form a second complex of antigen specific for ASCA and ASCA;
- (d) contacting said second complex with anti-immunoglobulin A antibody;
- (e) contacting said second complex with anti-immunoglobulin G antibody; and
- (f) diagnosing said individual as having IBD when the amount of first complex formed is greater than X,
- 20 the amount of IgA-containing second complex formed is greater than Y, or the amount of IgG-containing second complex formed is greater than Z,
- 25 and diagnosing said individual as not having IBD when the amount of first complex formed is less than X, the amount of IgA-containing second complex formed is less than Y, and
- 30 the amount of IgG-containing second complex formed is less than Z, wherein X, Y, and Z are independently selected to achieve an optimized clinical parameter selected from the group consisting of: sensitivity, specificity,

negative predictive value, positive predictive value and overall agreement,

provided that said method does not include histological analysis of neutrophils.

5 19. The method of claim 18, wherein said sample is a serum sample.

20. The method of claim 18, wherein said sample is a saliva sample.

10 21. The method of claim 18, wherein said antigen specific for ANCA is fixed neutrophils.

22. The method of claim 18, wherein said antigen specific for ASCA is yeast cell wall phosphopeptidomannan (PPM).

15 23. The method of claim 22, wherein said yeast cell wall PPM is prepared from strain ATCC #38926.

24. A highly efficient method of analyzing multiple samples for IBD, comprising the steps of:

(a) first assaying all samples for the presence or absence of ANCA;

20 (b) next assaying only ANCA-negative samples for the presence or absence of ASCA-IgA;

(c) next assaying only ANCA-negative and ASCA-IgA-negative samples for the presence or absence of ASCA-IgG,

25 wherein the presence of ANCA, ASCA-IgA or ASCA-IgG in a sample is indicative of IBD and

wherein the absence of pANCA, ASCA-IgA and ASCA-IgG is indicative of the absence of IBD.

25. The method of claim 24, wherein the presence of ANCA, ASCA-IgA and ASCA-IgG is determined using an immunoassay.

IBD FIRST STEP
CENTRAL COMPOSITE DESIGN

DOE EXP.	DANCA	ASCA-A	ASCA-G	50% IBD PREVALENCE (N=851)				15% CALCULATED PREVALENCE (N=851)				15% IBD PREVALENCE (N=277)						
				SENS.	SPEC.	ACC.	PPV	NPV	SENS.	SPEC.	ACC.	PPV	NPV	SENS.	SPEC.	ACC.		
1	0.5	10.0	20.0	96.3	13.6	55.7	60.5	78.1	96.3	13.6	26.0	16.4	95.4	100.0	15.7	28.5	17.5	100.0
2	0.5	10.0	60.0	95.2	14.8	55.7	52.6	74.7	95.2	14.8	26.9	16.5	94.6	100.0	17.0	29.6	17.7	100.0
3	0.5	30.0	20.0	96.1	14.4	55.9	53.8	77.9	96.1	14.4	26.7	16.5	95.4	100.0	16.6	29.2	17.7	100.0
4	0.5	30.0	60.0	94.1	16.3	56.3	54.0	75.6	94.9	16.3	28.1	16.7	94.8	100.0	18.7	31.1	18.0	100.0
5	1.5	10.0	20.0	81.3	64.6	73.1	70.4	76.9	81.3	64.6	67.1	20.8	95.1	81.0	58.3	61.7	25.4	94.5
6	1.5	10.0	60.0	77.1	75.4	76.3	76.4	76.1	77.1	75.4	75.7	35.6	94.9	76.2	69.8	70.8	31.1	94.3
7	1.5	30.0	20.0	78.5	70.8	74.7	73.6	76.1	78.5	70.8	72.0	32.2	94.9	73.8	65.1	66.4	27.4	93.3
8	1.5	30.0	60.0	69.5	86.1	77.7	83.7	73.2	69.5	86.1	83.6	46.9	94.1	64.3	82.1	79.4	39.1	92.8
9	1.0	20.0	40.0	82.2	67.3	74.9	72.2	78.5	82.2	67.3	69.5	30.7	95.5	85.7	61.2	65.0	28.3	96.0
10	1.0	20.0	40.0	82.2	67.3	74.9	72.2	78.5	82.2	67.3	69.5	30.7	95.5	85.7	61.3	65.0	28.3	96.0
11	0.5	20.0	40.0	94.9	16.3	56.3	54.0	75.6	94.9	16.3	28.1	16.7	94.8	100.0	18.7	31.0	18.0	100.0
12	1.5	20.0	40.0	74.1	83.5	78.7	82.3	75.7	74.1	83.5	82.1	44.2	94.8	71.4	78.3	77.3	37.0	93.9
13	1.0	10.0	40.0	85.0	61.0	73.2	69.3	79.7	85.1	61.0	64.6	21.8	95.8	90.5	55.7	61.0	26.8	97.0
14	1.0	20.0	40.0	80.6	57.4	69.2	66.2	74.1	80.6	57.4	60.9	25.0	94.4	85.7	62.6	66.1	29.0	96.1
15	1.0	20.0	20.0	85.2	57.7	71.7	67.6	79.0	85.2	57.7	61.8	26.2	95.7	85.7	51.9	57.0	24.2	95.3
16	1.0	20.0	60.0	81.8	67.5	74.7	72.2	78.1	81.8	67.5	69.7	30.8	95.5	83.3	63.8	66.8	29.2	95.5

Figure 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/10371

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 33/564, 33/569
US CL :435/7.21, 7.24, 7.31, 7.95; 436/506, 513

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.21, 7.24, 7.31, 7.95; 436/506, 513

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	COLOMBEL et al. Anti-Saccharomyces cerevisiae antibodies (ASCA) combined with antineutrophil cytoplasmic auto- antibodies (ANCA) in inflammatory bowel disease. Clinical and Experimental Immunology. 1998, Vol. 112, page 22, column 1, abstract no. 23, see entire abstract, especially last sentence.	1-25
Y	GIAFFER et al. Antibodies to Saccharomyces cerevisiae in patients with Crohn's disease and their possible pathogenic importance. Gut. 1992, Vol. 33, pages 1071-1075, see abstract.	1-25

 Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	*T*	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*&*	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
28 JULY 1999	31 AUG 1999

Name and mailing address of the ISA:US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DAVID SAUNDERS <i>D. Lawrence Saunders</i> Telephone No. (703) 308-0196
---	---

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/10371

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SAXON et al. A distinct subset of antineutrophil cytoplasmic autoantibodies is associated with inflammatory bowel disease. Journal of Allergy and Clinical Immunology. August 1990, Vol. 86, No. 2, pages 202-210, see abstract and page 208, column 1.	1-25
Y	SENDID et al. Specific antibody response to oligomannosidic epitopes in Crohn's disease. Clinical and Diagnostic Laboratory Immunology. March 1996, Vol. 3, No. 2, pages 219-226, see abstract.	22-23

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/10371

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, BIOSIS, CAS:

L1 ANCA OR ANCAS
L2 ANTI(w)NEUTROPHIL
L3 ANTINEUTROPHIL OR ANTINEUTROPHILS
L4 CYTOPLASMIC OR CYTOPLASMICS
L5 AUTO(w)ANTIBODY
L6 AUTOANTIBODY OR AUTOANTIBODIES
L7 ANTIBODY OR ANTIBODIES
L8 (L2 OR L3)(w)L4(w)(L5 OR L6 OR L7)
L9 L1 OR L8
L10 ASCA OR ASCAS
L11 ANTI(w)SACC
L12 ANTI(w)SACCHAROMYCES
L13 ANTISACCHAROMYCES
L14 SACCHAROMYCES
L15 CEREVISIAE
L16 S(w)L15
L17 L14(w)L15
L18 L16 OR L17
L19 L5 OR L6 OR L7
L20 L18(5A)L19
L21 L10 OR L11 OR L20
L22 L9 AND L21
L23 L9 OR L21